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The Ultraviolet Absorption Spectrometry (UVAS) and Liquid Atomic Emission Spectrometry (LAES) for Oceanographic Analysis Systems project was a four-phase project that covered over 20 months from May 22, 1992 to February 10, 1994. Under contract with the Naval Research Laboratory at Stennis Space Center, Mississippi, Biotronics Technologies, Inc., worked to design, build, and test an on-line spectrophotometer capable of real-time, reagentless chemical analysis of ocean and bay waters. More specifically, the project had three objectives. First, to determine spectral parameters of nutrients and selected metals, first in pure distilled water and then in ocean and bay water, and to use this information to predict analyte concentrations and determine design parameters for the deliverable instrument. Second, to design, construct, and test the hardware and software for the deliverable instrument. And finally, to test the instrument on board a Navy test vessel in actual field conditions and determine the accuracy of analyte concentration predictions.

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ULTRAVIOLET ABSORPTION SPECTROMETRY (UVAS)

AND

LIQUID ATOMIC EMISSION SPECTROMETRY (LAES)

FOR

OCEANOGRAPHIC ANALYSIS SYSTEMS

FINAL REPORT
Naval Research Laboratory
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APPENDICES

1. PROJECT SUMMARY

The Ultraviolet Absorption Spectrometry (UVAS) and Liquid Atomic Emission Spectrometry (LAES) for Oceanographic Analysis Systems project was a four-phase project that covered over 20 months from May 22, 1992 to February 10, 1994. Under contract with the Naval Research Laboratory at Stennis Space Center, Mississippi, Biotronics Technologies, Inc., worked to design, build, and test an on-line spectrophotometer capable of real-time, reagentless chemical analysis of ocean and bay waters. More specifically, the project had three objectives. First, to determine spectral parameters of nutrients and selected metals, first in pure distilled water and then in ocean and bay water, and to use this information to predict analyte concentrations and determine design parameters for the deliverable instrument. Second, to design, construct, and test the hardware and software for the deliverable instrument. And finally, to test the instrument on board a Navy test vessel in actual field conditions and determine the accuracy of analyte concentration predictions.

During Phase I of this project, all analytes of interest were scanned with either ultraviolet/visible/near infrared absorption and/or atomic emission spectrometers to determine their most promising spectra for later multi-component analysis. Phase II built on the first phase by scanning the analytes in complex solutions combining more than one analyte at varying concentrations. Initial multi-component studies were completed in a distilled water background followed by experiments with simulated ocean water background. This phase determined the analytical potential and optimal spectrometric method for each analyte.

Moving to Phase III meant moving from laboratory analysis work with existing instrumentation to designing, manufacturing, testing, and calibrating a new prototype instrument. The Oceanographic Hybrid Absorption/Emission Spectrometer (OHAES), the new prototype instrument, was a highly modified, improved version of an earlier Hybrid Absorption/Emission Spectrometer (HAES) developed and built by Biotronics Technologies for a NASA SBIR project. After completing in-house testing and calibration, the OHAES was shipped to the U.S. Naval Academy in Annapolis, Maryland, for Phase IV field testing.

All Phase IV testing was accomplished on the Naval Academy's YP-686, a yard patrol craft used for a variety of oceanographic testing by the academy's oceanography department and the National Oceanographic and Atmospheric Administration (NOAA). Four separate cruises in the Chesapeake Bay were completed, during which the OHAES instrument was operated and bay water samples were collected for laboratory analysis and comparison to the OHAES concentration predictions. In addition, the laboratory concentration predictions were used to update the original calibration with actual field samples and thereby incorporate the actual background into the calibration algorithm.

This report covers all four phases of the project. Before getting into the details of the experimental work, the basic spectrometric concept of molecular absorption and atomic emission are explained in Section 2. Next, Section 3 more fully reviews the specific project objectives and the chemical analysis and technical approaches to achieve those objectives. The instrumentation used throughout the project as well as the final deliverable instrument will be described in Section 4. Detailed discussion of the analytical methods and chemometric techniques used to transform the spectral information into analyte concentrations is covered in Section 5. Section 6 summarizes the results for each phase of the project. The general findings and conclusions are explained in Section 7. Finally, avenues for follow-on work to improve the delivered instrument or to modify it for alternate applications are outlined in Section 8.

2. SPECTROMETRIC CONCEPTS

In order to maximize the number of analytes whose concentrations can be predicted by the delivered instrument using reagentless spectroscopy, both molecular absorption and liquid atomic emission technologies have been studied and incorporated into the OHAES. This section briefly reviews the principles underlying these technologies.

2.1 Molecular Absorption Spectrometry

Absorption spectrometry takes advantage of a phenomenon in which some fraction of light directed at a solution is absorbed by the compounds in the solution while the balance of the light passes through the solution. The light that passes through the solution is divided into discrete wavelengths by a spectrograph. Then, the amplitude of light at each wavelength is detected by a photodiode detector array. The important features of absorption spectra are the position (wavelengths) and intensity of the spectral lines produced. These features result in a signature that can be used to identify a substance.

The three basic components of an absorption spectrometer are a light source, a sample cell, and a light measurement device. See Figure 2-1 for a layout of the basic components of an absorption system. The OHAES light source is a xenon flash lamp that provides light from the ultraviolet through the visible and near infrared wavelengths. This lamp was selected because it provides light in the ranges that Phase I laboratory testing indicated were optimal for the analytes of interest. The lamp's flash capability made it possible to design the lamp assembly without any moving parts.

The light measurement device must be able to discern the intensity of light at specific wavelengths. For the OHAES, a dispersive spectrograph divides the light coming from the flow cell into discrete wavelengths (from approximately 200 to 800 nm). This dispersed light is detected by a silicon photodiode array that has 1,024 separate photodiodes to collect the light. The intensity of light at each photodiode is then transferred to a computer data file.

The OHAES has two absorbance sample cells (flow cells) that are designed to allow constant flow or to be used for discrete samples. The main characteristic of the flow cells is that they provide a distinct path length the light must travel through the solution being tested. Because the OHAES has two absorbance flow cells, two different path lengths may be used at one time. The absorbance flow cells have been designed so that the path lengths can be varied from 2 mm to 100 mm. The OHAES was initially calibrated with 25 mm and 100 mm path length flow cells. Additional path lengths may be used by changing the hardware. The range in path lengths directly corresponds with the range of analyte concentrations being studied, especially those of nitrate and nitrite.

Having two flow cells with two different path lengths that can be used simultaneously will be especially helpful when there is no information regarding the possible actual concentration of the analytes. In addition, absorbance comparisons between the two flow cells can be used to compute concentrations.

Theoretically, the relationship between the degree of absorption of a solution and the concentration of analytes in the solution is described by the Beer-Lambert Law[9]. This law takes into account other parameters that may affect the absorbance of a substance. The law is stated below:

$$A = abc$$

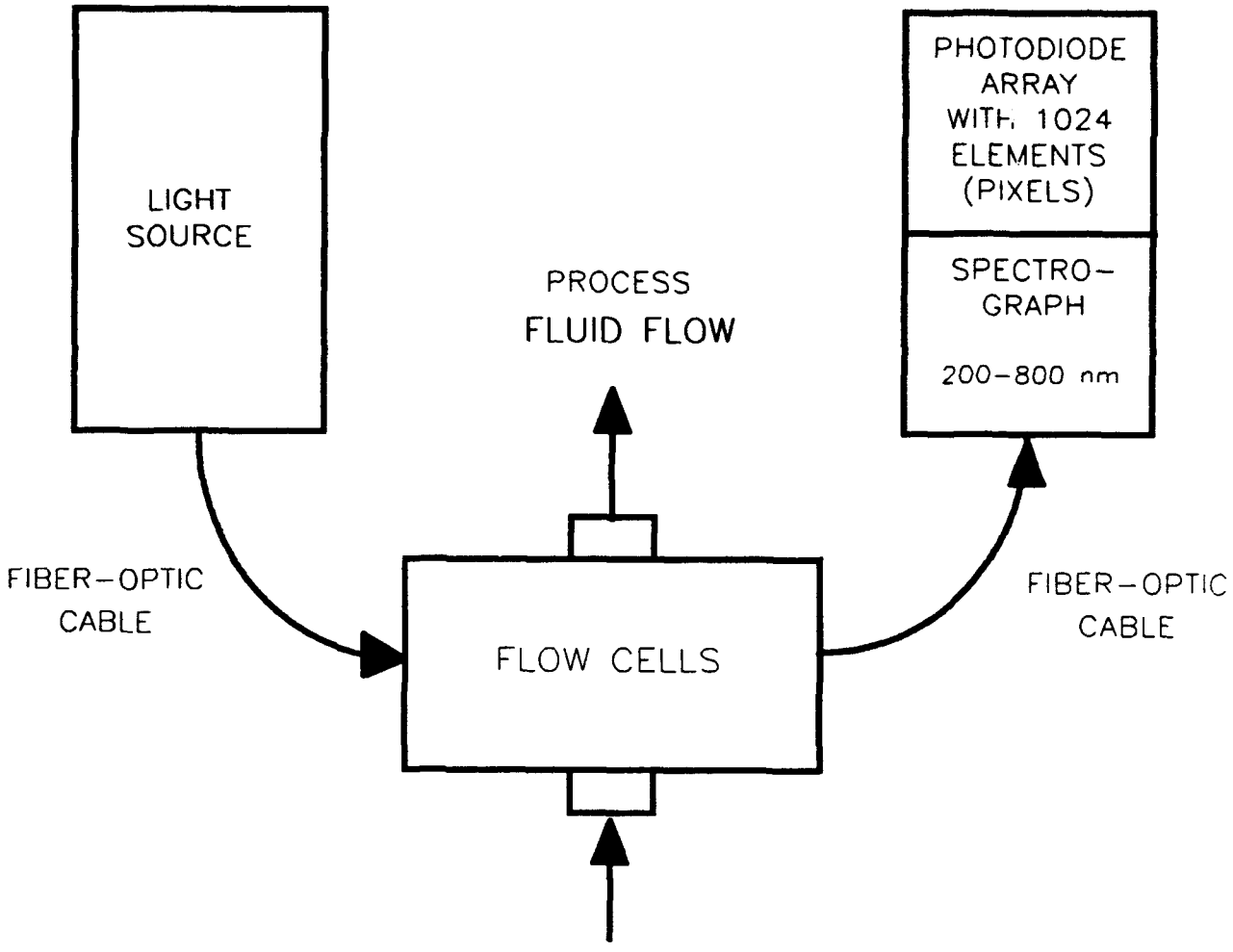

FIGURE 2-1. ABSORPTION BASICS

where

A = the total amount of light absorbed;
a = the absorption coefficient defining the absorptivity of the medium;
b = the length of the absorption light path; and
c = the concentration of the solution.

From the Beer-Lambert Law, we see a direct relationship between the absorbance and the concentration of an analyte in solution. However, a single analyte in a perfectly transparent solvent is an unusual occurrence in absorption spectrometry. Because of background interference and the overlapping of absorbance wavelengths of the various components in solution, the chemometrics necessary to solve for analyte concentrations become complex. The most effective means to solve this problem is to use rotated principal components analysis. The analytical methods used to predict analyte concentration are more fully discussed in Section 5.

To initially measure the total amount of light absorbed (A), the intensity of the absorbed light (i.e., the intensity of light after passing through the absorbing solution) is compared to the intensity of incident light (i.e., the intensity of light after passing through the same optical path containing a non-absorbing solution). The absorbance is calculated using the following equation[9]:

$$A = -\log(I_o/I_i)$$

where

A = absorbance;
I_o = intensity of absorbed light; and
I_i = intensity of incident light.

A standard laboratory absorbance instrument normally produces two beams of light so that both the absorbed and incident light intensities can be measured simultaneously. The OHAES, however, compares the absorbed light intensity to a previously stored spectrum of the intensity of light passing through distilled water in the same flow cell. This stored spectrum of distilled water is referred to as the instrument standard.

A plot showing the intensity of incident and absorbed light in a typical sample is shown in Figure 2-2. Next, the computed absorbance based on those light intensities is given in Figure 2-3. Notice the spiked nature of the "raw" light from the xenon flash lamp. However, because the light intensity is very stable, the computed absorbance is a smooth curve.

2.2 Liquid Atomic Emission Spectrometry (LAES)

Liquid Atomic Emission Spectrometry (LAES) is a new variation of an old technology. The basic theory of atomic emission is based on the change of state of the electrons of an atom[13]. When an electron moves from one energy level to another, it must absorb or emit the exact amount of energy required to bring it from the initial to the final state. Because each element is unique, the energy required for these changes of state is unique to each element. After an atom is excited to a higher energy level by an input of energy (for LAES, an electrical spark/arc combination is used), the atom will return to an intermediate, lower energy state or its ground energy state with a release of discrete radiant energy called photons. The wavelength of the photons released is characteristic of the element and can be used to identify and quantify the element.

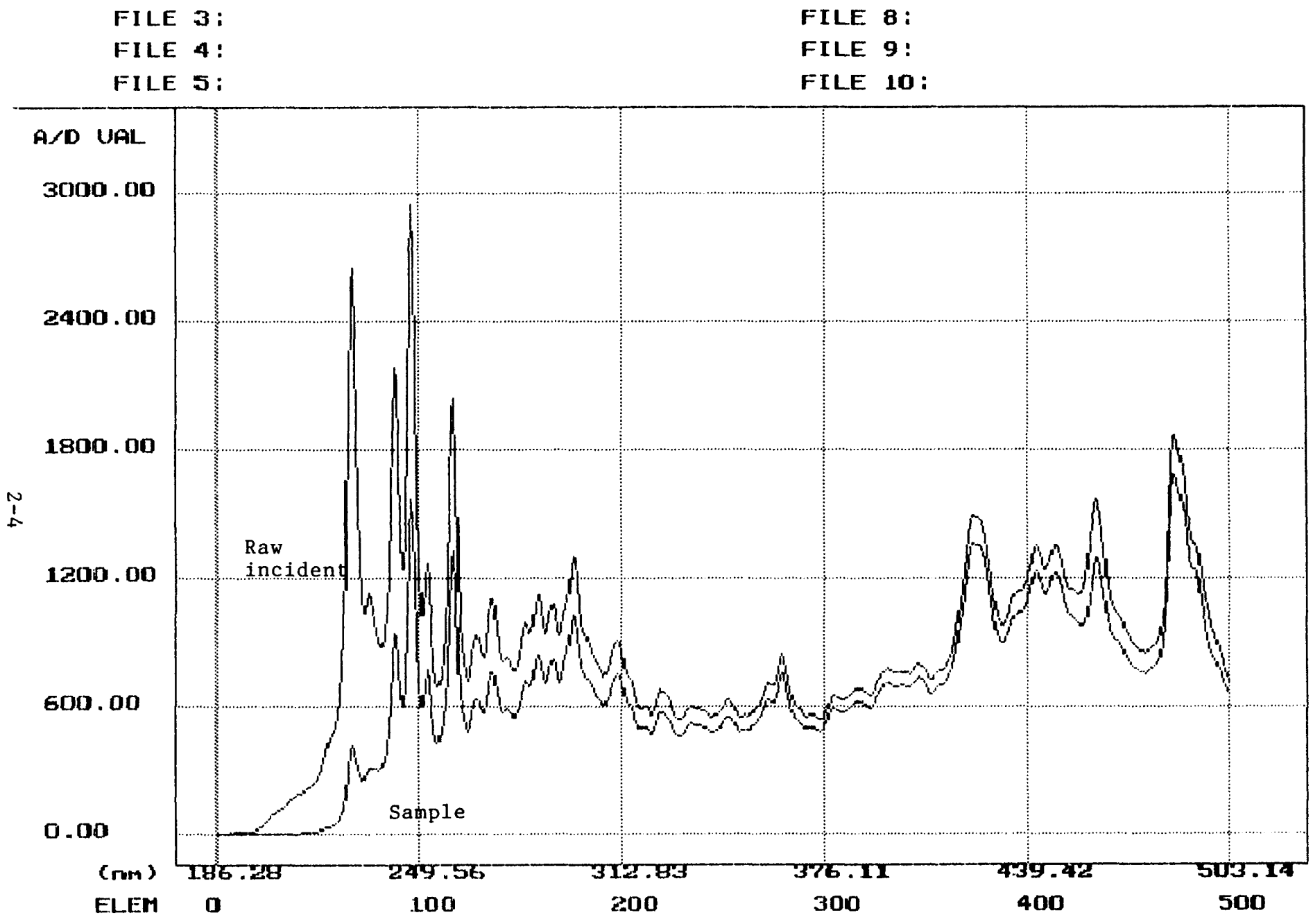

Figure 2-2. "Raw" incident and absorbed light

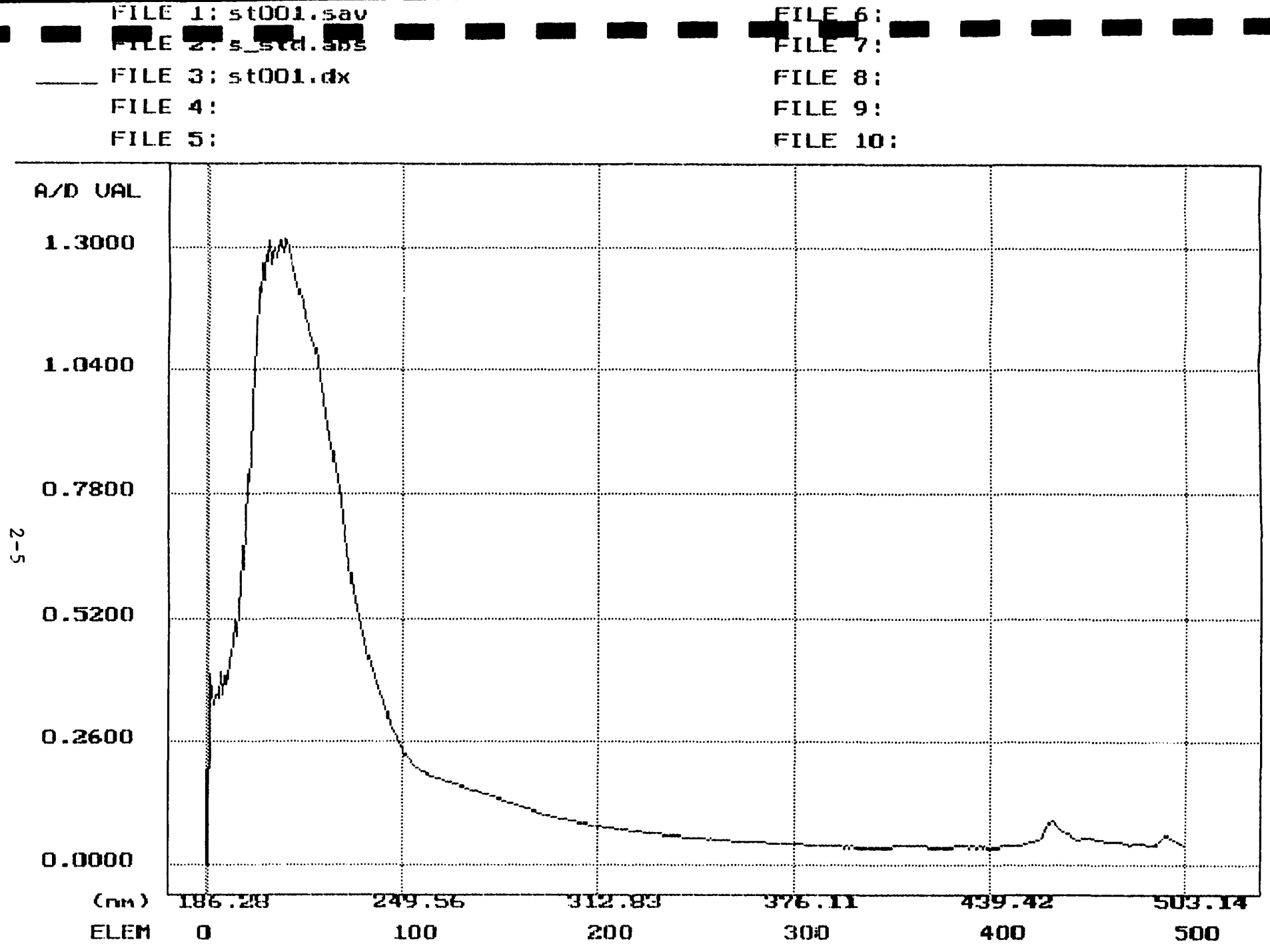

Figure 2-3. Computed absorbance

Classically, atomic emission is performed in the laboratory using a flame for excitation of the substance being studied. More commonly used today as a method of atomic emission excitation is the inductively coupled plasma (ICP) formed by a magnetic field[10]. However, for an on-line, continuously operating instrument, the technology had to be applied to a liquid process flow. By using a spark/arc combination originated by a large voltage potential across a pair of gold electrodes directly in the solution in the flow cell, the OHAES generates a spectrum characteristic of the elements in the solution.

The OHAES design incorporates an emission flow cell through which a side stream of the process solution flows. This flow cell contains gold electrodes that are the source of the spark/arc excitation. A fiber-optic cable carries the light emitted in the flow cell back to a spectrograph, the same one as used for absorbance measurements, to be dispersed and then detected by the photodiode array used for absorbance measurements. Because of the high-voltage pulses necessary to excite the atoms of the solution and therefore the potential electrical interference with other electronic components, the emission power supply is physically separated from the main instrument electronics. The only connections are via fiber-optic cables. See Figure 2-4 for a layout of the major components of the emission system.

Theoretically, the intensity of the light emitted at a particular wavelength is directly related to the concentration of the element with that characteristic emission frequency[22]. However, variations in the control of energy input to the system, as well as interference among elements that emit at nearby wavelengths, complicate the analysis of the spectra. Many laboratory atomic emission instruments make use of a reference element of known concentration to aid in normalizing the spectra[22]. For an on-line field instrument, it was not considered desirable to add anything to the process flow. Extensive mathematical experimentation showed that the best way to normalize and minimize variation was to sum the total energy (i.e., the total intensity of light emitted across the entire spectrum) and to divide the intensity at each wavelength by this total. Figures 2-5 and 2-6 show a raw emission spectrum and that same spectrum after it has been normalized to total energy. Visually examining these two curves shows them to be very similar; however, repeatability studies that compared the coefficient of variation of the two types of spectra indicated that the normalized spectra had less than half the variation of the raw spectra.

After the emission spectra are normalized, the intensity at specific wavelengths is used to predict elemental concentrations. In simple solutions, without background or varying concentrations of other analytes, simple linear relationships exist between the normalized intensity at one major wavelength and the given analyte concentration. Figure 2-7 shows an example of this relationship for potassium, which is known to emit at 769.90 nm. Here the normalized intensity of emissions at that wavelength track directly with the increasing potassium concentration. As background solutions are added or varied, the algorithms necessary to predict analyte concentrations require multiple-variable stepwise regression to eliminate the effects of interference. Section 5 provides detailed descriptions of these analytical techniques.

In order to obtain a "clean" spectrum given a low signal-to-noise ratio, many parameters of the LAES system must be fine-tuned. These various parameters include the auxiliary voltage spark gap, the electrode gap (within the flow cell), the current, the period and burn time, and the number of sparks/arcs within each "flash." As each of these parameters is varied, the spectral output varies; therefore, the parameters must be carefully adjusted to provide optimal performance.

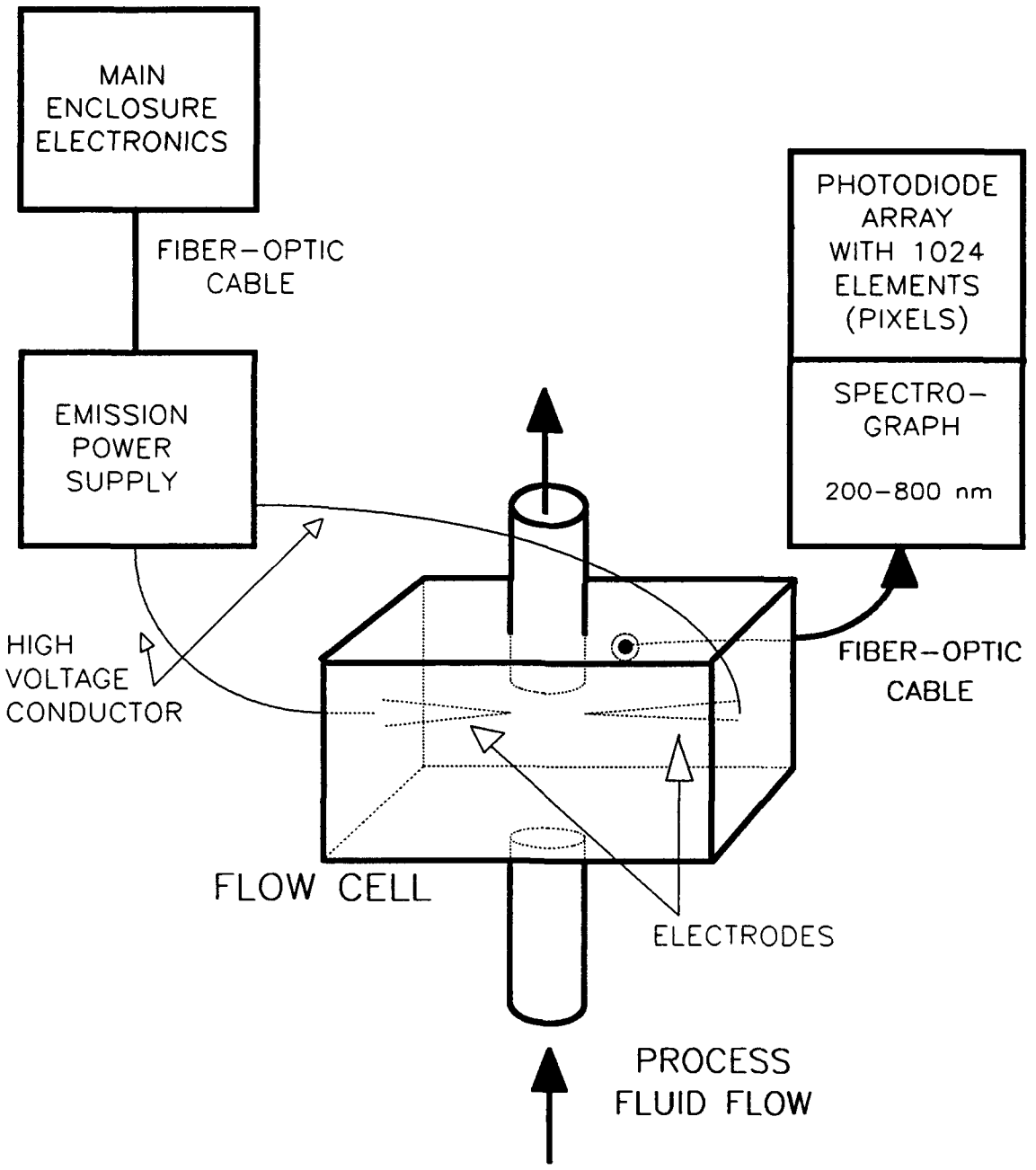

FIGURE 2–4. EMISSION BASICS

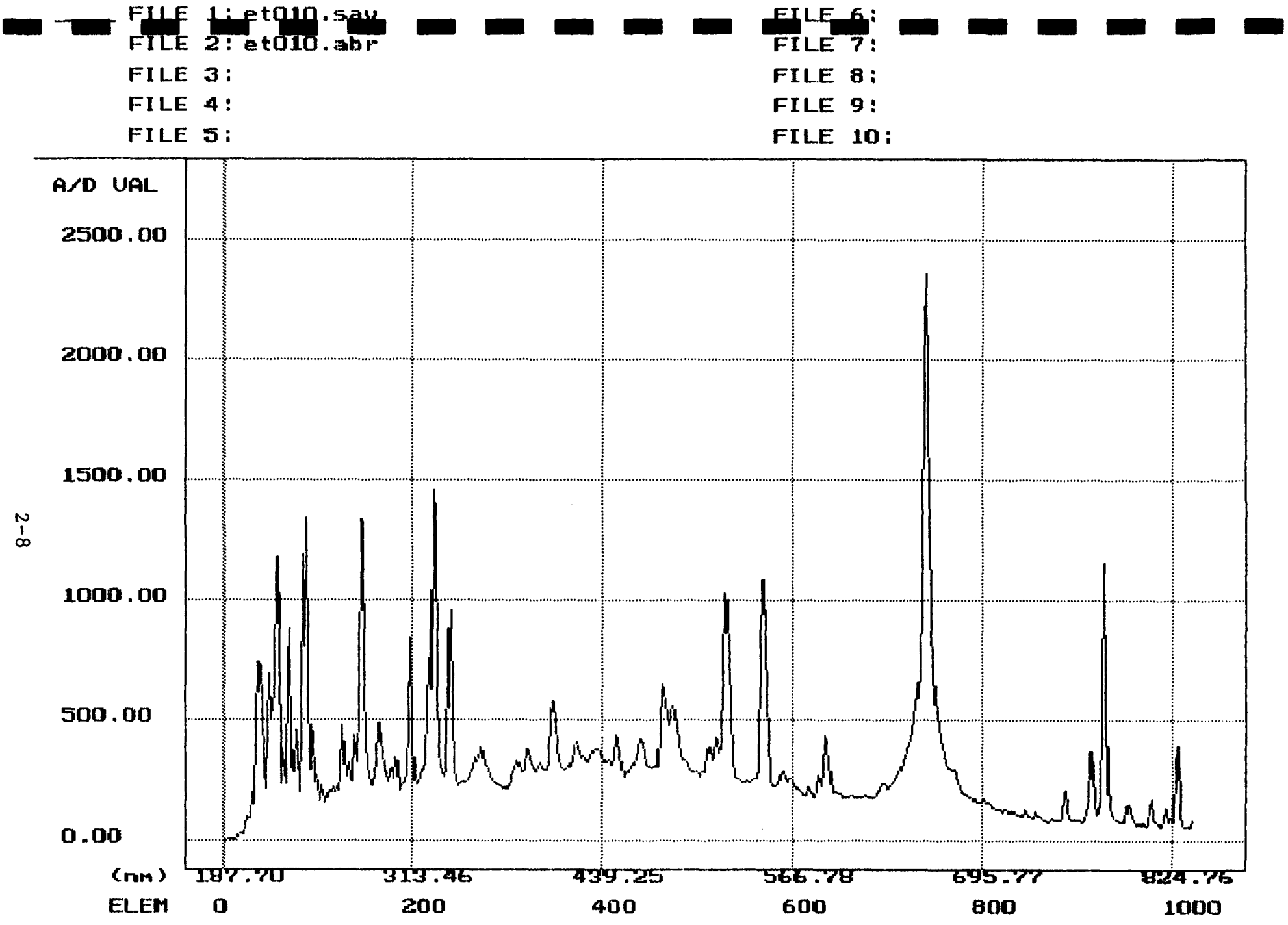

Figure 2-5. Raw liquid atomic emission spectrum

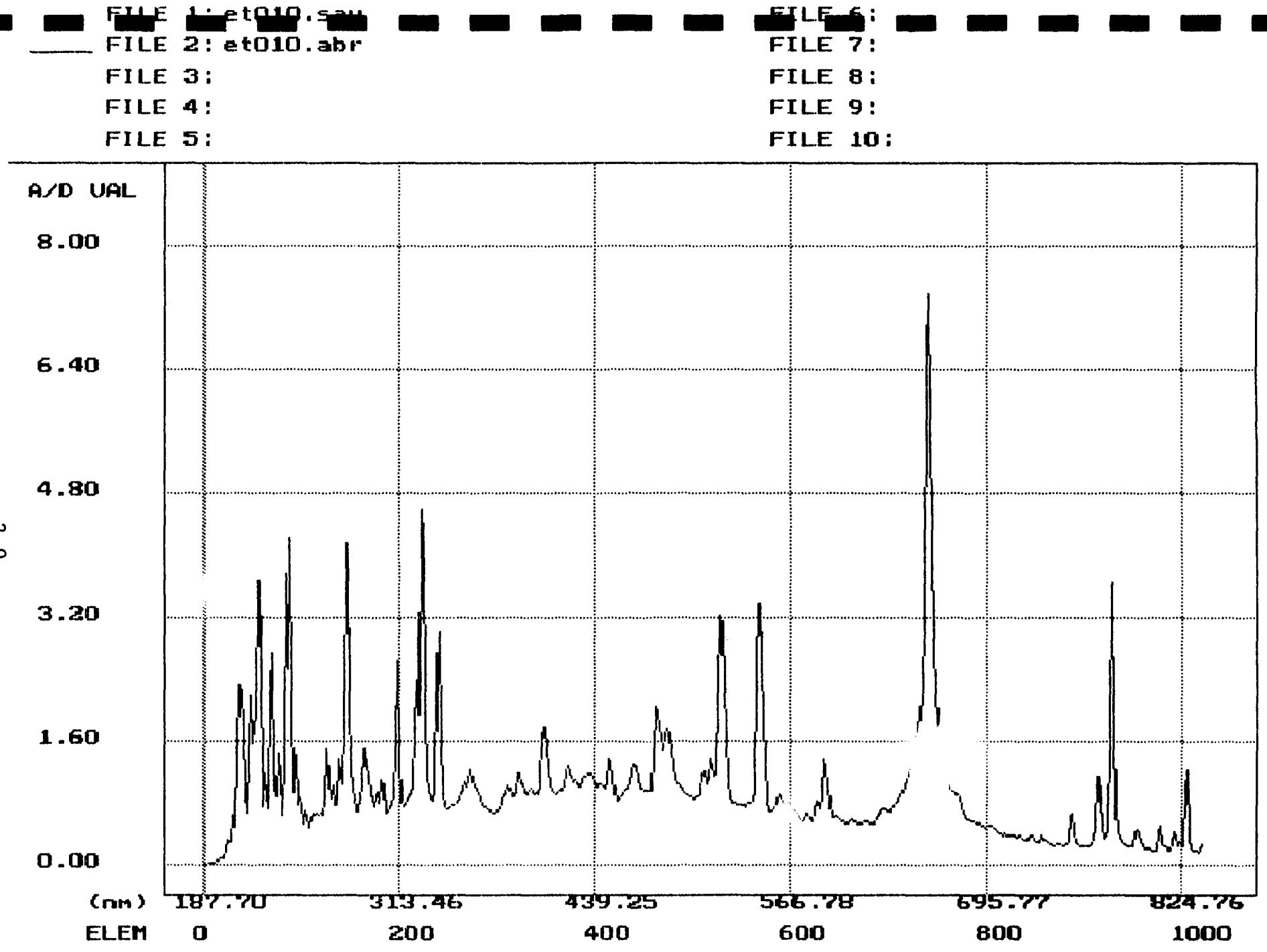

Figure 2-6. Normalized liquid atomic emission spectrum

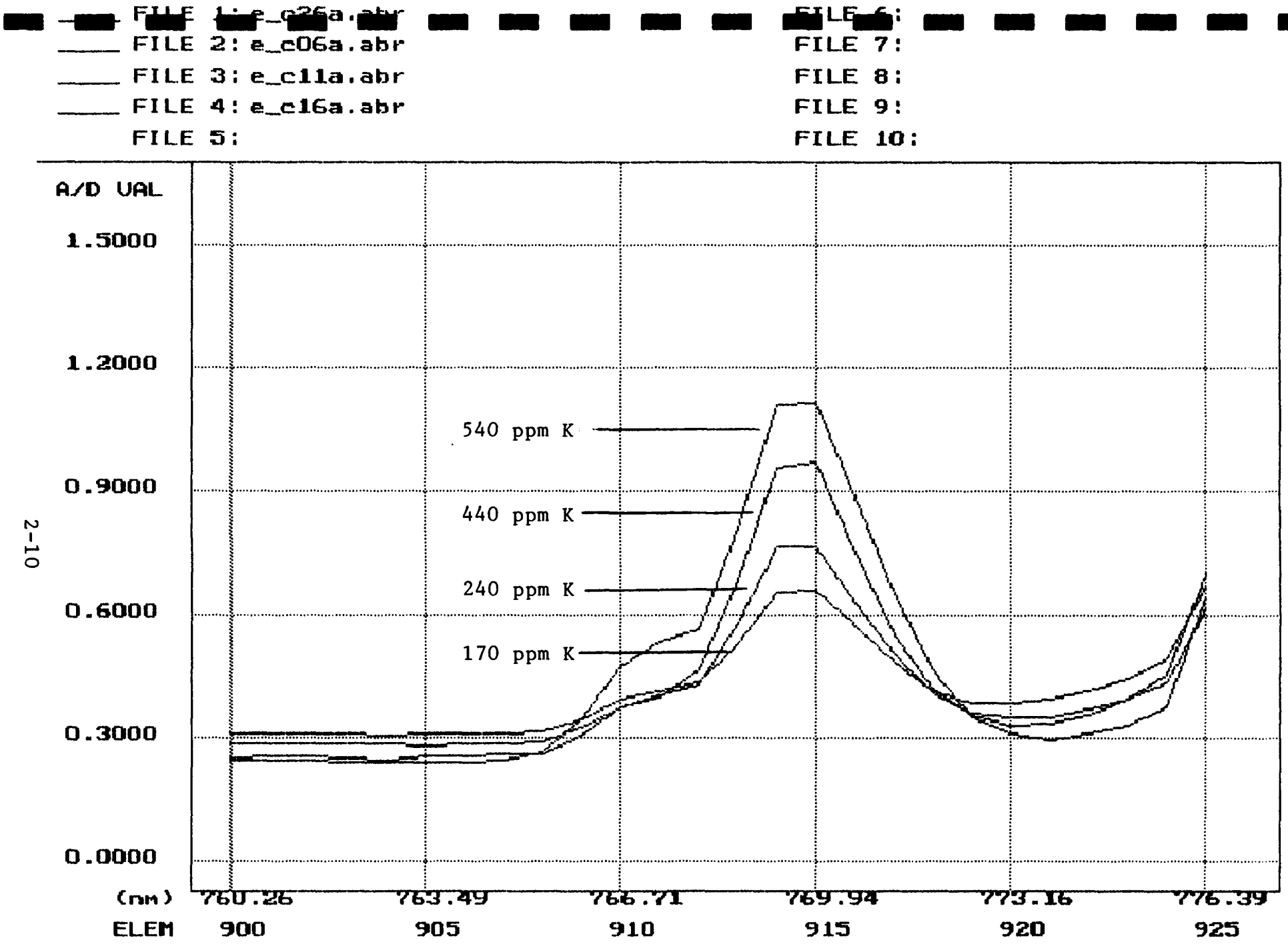

Figure 2-7. Potassium emission spectra

The OHAES system's performance is considered optimal when the signal-to-noise ratio is maximized without saturating the photodiode array. This is difficult to achieve because the light emitted at some wavelengths is many orders of magnitude greater than the light emitted at other wavelengths. An experienced and well-trained operator is essential to successful operation of the LAES system. In addition, because the LAES power supply can generate extremely high voltages (up to 60,000 volts) it must be operated only by a highly skilled and knowledgeable person.

3. PROJECT OBJECTIVES AND APPROACHES

The central objective of this research project was the development of technology for real-time, reagentless chemical analysis based on Ultraviolet-Visible Absorption Spectrometry (UVAS) and the new Liquid Atomic Emission Spectrometry (LAES). The project focused on the development and delivery of an on-line spectrometer capable of providing such chemical analysis information for ocean or bay waters. The focus of this research was to determine whether UVAS and LAES technologies can be developed to provide on-line measurements of analytes such as nitrate, nitrite, phosphate, silica, and trace metals that may be of interest to the Navy's oceanographic research programs.

The original proposal divided the approach to this project into five phases as outlined below.

Phase I	-	Individual Analyte Analysis (Laboratory)
Phase II	-	Multiple Analyte Analysis (Laboratory)
Phase III	-	New Instrumentation System Development and Simulated Field Test
Phase IV	-	Ocean Field Test
Phase V	-	Development and Delivery of an Operational Instrumentation System to the Navy

Phases I-IV have been funded by the Naval Research Laboratory at Stennis Space Center and are complete at this point. Phase V funding should be addressed based on the results of Phase IV testing and the availability of additional environment research funding.

One of the key objectives of this project was to develop a real-time, reagentless chemical analysis system. The majority of approved, accurate chemical analysis technologies in use today require the collection of samples for later, time-intensive analysis in a remotely located chemical laboratory. This off-line procedure not only severely limits the amount of oceanographic chemical data collected, but it also greatly reduces the quality of the data because of changes that can occur to the sample's chemical and biological make-up. There is no assurance that a laboratory measurement performed several hours or possibly days after sample collection will accurately reflect the true chemical analysis that existed at the time of sample collection. In contrast, the new, on-line, reagentless OHAES system can provide for high speed, high volume collection and analysis of quality spectrometric data in an ocean or bay environment. In addition, because of its use of primary (natural) spectra, no chemical reagents are required to complete an analysis. A reagentless system is cheaper to operate and, in addition, does not add potential hazardous chemicals to the sample water.

3.1 Chemical Analysis Objectives and Approach

The first two phases of the project were based on laboratory analysis of various components of ocean water. The major objective of the Phase I work activity was to generate basic spectra in the appropriate concentration ranges on absorption and/or atomic emission instrumentation to determine the feasibility of spectral analysis of those ocean water components (analytes). If basic, reagentless spectra (i.e., absorption or emission spectra for the compound without any added reagents) do not exist or are very weak for a given analyte in isolation (i.e., in distilled water), then there is very little possibility the analyte will have usable spectra in a multicomponent solution. The following analytes were studied in Phase I:

1. Nitrate
2. Nitrite
3. Ammonia
4. Silica
5. Iron
6. Molybdenum
7. Zinc
8. Copper
9. Calcium
10. Potassium
11. Magnesium

The analytes above were studied on the Perkin Elmer Lambda 9 Spectrophotometer that can scan absorbance from the ultraviolet through the near infrared. Those analytes without promising absorbance spectra were then studied on the prototype LAES system under development for NASA. Results of these studies are explained in Section 6.

Phase II of the project took this research one step further by studying in multicomponent solutions each of the analytes that produced useable spectra in Phase I. Initial studies were based on solutions prepared in a distilled water background; subsequent studies were completed with the analytes in a simulated ocean background. A commercially available ocean imitation, "Instant Ocean," was used for the ocean background.

In the multicomponent solution, the spectra of the analytes interfere with each other and could block detection of the analytes in some cases. The objective of this phase was to determine which analytes would be affected by this interference. In addition, the ocean background with its high salinity could cause changes in the chemical behavior of the ions in solution and therefore could cause changes in the spectra of the analytes.

Spectral absorbance and emission studies for Phase II were completed on a prototype version of the NASA HAES, which served as a baseline for the design of the deliverable OHAES system.

3.2 Hardware and Software Development Objectives and Approach

After completing the chemical experiments defining the type of spectra, wavelengths, and dynamic range required for analysis of ocean and bay waters, the OHAES system was designed in detail. Phase III of this project centered on development of the hardware and software necessary to meet the chemical analysis objectives. The OHAES is a highly modified version of an earlier HAES instrument designed for and delivered to NASA for monitoring nutrients in hydroponic plant solutions in the Biomass Production Chamber at the Kennedy Space Center. Fresh design concepts have been incorporated into the new instrument that adapt it to predicting analyte concentrations in ocean and bay waters as well as to operation on board a Navy vessel. In addition to optimization of mechanical design and the manufacture of parts, several software programs were designed, written, and debugged to provide an optimal user interface as well as precise and accurate analyte concentration predictions.

The objective of the hardware design, manufacture, and test effort was to design and build a system capable of achieving the chemical analysis objectives explained above within the given project constraints of cost and schedule. The OHAES instrument consists of two major subsystems. The brain of the instrument consists of the "Analyzer," which includes the computer, video monitor, keyboard and circuit boards that control the instrument. The heart of the instrument is the "Optograph," which includes the spectrograph, photodiode array detector, power supplies, absorption light source, and flow cells. The original proposal suggested designing a towable, submersible

optograph that would interface with an on-board analyzer. The intent was to use a tow body that would be supplied by the Navy; however, available tow bodies were too small to fit this prototype version of the OHAES. While subsequent models of the OHAES could be significantly miniaturized, the expense of doing so is necessarily delayed until after the performance of the system is verified in Phase IV testing. In addition, the planned test vessel, the YP-686 at the Naval Academy, has a water intake approximately 2 meters below the stern. This intake would provide a water flow sufficient to test the capability of the OHAES to analyze the chemical composition of the near-surface bay water. By modifying the contract and proceeding to build a non-towable OHAES system, the cost and schedule criteria of the project were maintained while still achieving the basic objective of testing the feasibility of using absorbance and emission spectrometry to perform ocean/bay water analysis.

To meet the OHAES project objectives, the NASA HAES design was improved and modified as necessary to meet this new application. A variety of specific improvements were included in the OHAES design. In order to increase stability from reading to reading, the temperature control system for the spectrograph/array assembly was modified to allow for more precise control and was designed with a significantly smaller heat sink. The absorbance flow cell system was modified to include two flow cells. Having two flow cells allows the operator to study two different absorbance path lengths simultaneously as well as opens the option for comparing the two absorbances. The absorbance flow cells were also redesigned to allow for easier cleaning and maintenance without disturbing the optical path. The emission power supply was modified to ensure reliable emission excitation in highly conductive ocean waters. The entire system was packaged in one standard 19-inch rack with removable components for ease in transportation and installation.

In addition to design, manufacture, and test work conducted during Phase III of the project, software design and development was required. The main objective of the software design was to write a user-friendly interface, based on object-oriented software that could be easily modified and updated. A second software objective was to update the calibration method and data storage procedures to ensure a complete record of data was saved and that calibration techniques could be accurately completed. Some of the software from the NASA HAES was used in the OHAES system, but a majority of the software was new because the user interface was changed dramatically to meet the objective of being "user friendly." The new OHAES instrument operation software is based on pull-down menus that are easily controlled through the use of a mouse. Data storage objectives were achieved through the development of a new type of data file: a composite binary file. This file combines raw binary spectral data with instrument and sample information such as sample concentrations (if known), the instrument standard and instrument settings. This composite file significantly improves the quality of stored data by making the files more complete. However, the new file required quite a bit of additional programming to incorporate it into data analysis software.

3.3 In-House and Field Testing Objectives and Approach

After completing manufacture, electrical continuity testing, and stability testing of the OHAES system, the instrument was performance tested and calibrated "in-house" at Biotronics Technologies based on laboratory prepared samples that use Instant Ocean as a background. The objective of the in-house testing was to ensure the previous spectral studies completed in Phases I and II could be repeated on this new instrument. In addition, the in-house testing was used to calibrate the instrument based on laboratory samples, a calibration learning set, and a separate test set. The initial calibration algorithms would be stored and evaluated later during the field tests.

Phase IV commenced with shipment of the OHAES to the U.S. Naval Academy at Annapolis, Maryland for testing on the YP-686, a yard patrol craft used for oceanography experimentation. Dr. John Foerster of the academy's Oceanography Department and Mr. Ken Sabel of the Hendrix

Oceanography Laboratory were instrumental in helping Biotronics Technologies achieve the field testing objectives.

Four cruises were planned for Phase IV testing. Due to time limitations and restrictions on the YP-686 test vessel, testing in the open ocean was not feasible. Therefore, the intent was to cover a wide range of waters in the Chesapeake Bay by cruising up rivers and into the center of the bay. During the first two cruises, the original analyte calibrations were tested to determine whether they were producing suitable analyte concentration predictions. In addition, water samples were collected for independent laboratory analysis to determine actual analyte concentrations. Next, the original calibration algorithms were updated by inputing the spectra from the actual bay water samples. This incorporated the background of the bay into the calibration algorithms and was expected to significantly improve the calibrations. Then, these updated calibrations were tested on subsequent cruises. Again, for comparison, water samples were collected and sent to an independent laboratory for analysis.

4. INSTRUMENTATION

This section will review the instrumentation used throughout the four phases of the project, including a detailed description and schematics of the final delivered OHAES system.

4.1 Perkin Elmer Lambda 9 Spectrophotometer

Phase I absorbance studies were conducted on the Perkin Elmer Lambda 9 Spectrophotometer. The Lambda 9 is a dual-beam, dual-monochromator, ultraviolet/visible/near infrared spectrometer useable in a wide range of applications. Sample cells of various path lengths from 1-100 mm may be used in the analysis. Within the Lambda 9, there are two monochromators in series, each containing two automatically changing gratings. The Lambda 9 has two light sources: a deuterium lamp for the ultraviolet range and a tungsten-halogen lamp for the visible to near infrared range. A side-window photomultiplier is the detector in the ultraviolet/visible range. A lead-sulfur detector is used in the near infrared. The total useable wavelength range for the Lambda 9 is 185-3200 nm. Due to physical limitations with the planned ocean/bay water application, however, Phase I study was restricted to 220-1800 nm. Wavelengths below 200 nm are not useable except in a vacuum. Above 1800 nm the absorbance of water causes too much interference, making the data unusable.

4.2 Liquid Atomic Emission Spectrometer Prototype

Emission studies for Phase I were completed on a prototype Liquid Atomic Emission Spectrometer (LAES). This prototype was designed and built by Biotronics Technologies as the basis for the emission portion of NASA's HAES system. A block diagram depicting the prototype system is shown in Figure 4-1. Because of the high salinities expected during testing of the OHAES, the emission power supply had to be upgraded to provide the power necessary to generate the arc/spark excitation necessary for the emission to occur. Without sufficient power, the high conductivity of ocean or bay water causes the arc/spark current to short out without producing an emission excitation spectra.

After the emission power supply, the emission flow cell is the second most critical component of the LAES system. A diagram of the emission flow cell is depicted in Figure 4-2. For safety reasons, because of the high electrical voltages generated within the flow cell, black Delrin, an acetal plastic that is fairly stable under high temperatures and has low water absorption, was selected as the flow cell body material. The arc/spark excitation current is passed through the flow cell across 18 kt gold electrodes with a 1 mm gap between them. The emission spectrum is captured by a fiber-optic cable. In addition, a viewing window allows the operator to qualitatively evaluate the emission arc/spark. A variety of materials were studied as possible electrode material, but 18 kt gold was found to produce the least interference of adding extra peaks to the spectra, and it had excellent electrical and temperature conductivity. Ideally, pure gold electrodes would have less interference with the observed spectra, but cost limited the project to the use of 18 kt gold.

4.3 NASA Hybrid Absorption/Emission Spectrometer (HAES)

The NASA HAES was designed and manufactured by Biotronics Technologies for analysis of plant nutrient solution by collecting both molecular absorption spectra and liquid atomic emission spectra. This instrument was used for Phase II experimentation for this project. For absorbance studies, light from a single-beam xenon flash lamp is transmitted to the absorbance flow cell via fiber-optic cables. The absorbance flow cell was modified to allow for studies using various path lengths. For liquid atomic emission studies, fluid in the emission flow cell is electrically excited by a spark/arc combination, producing a voltage potential across the submersed electrodes. This excitation

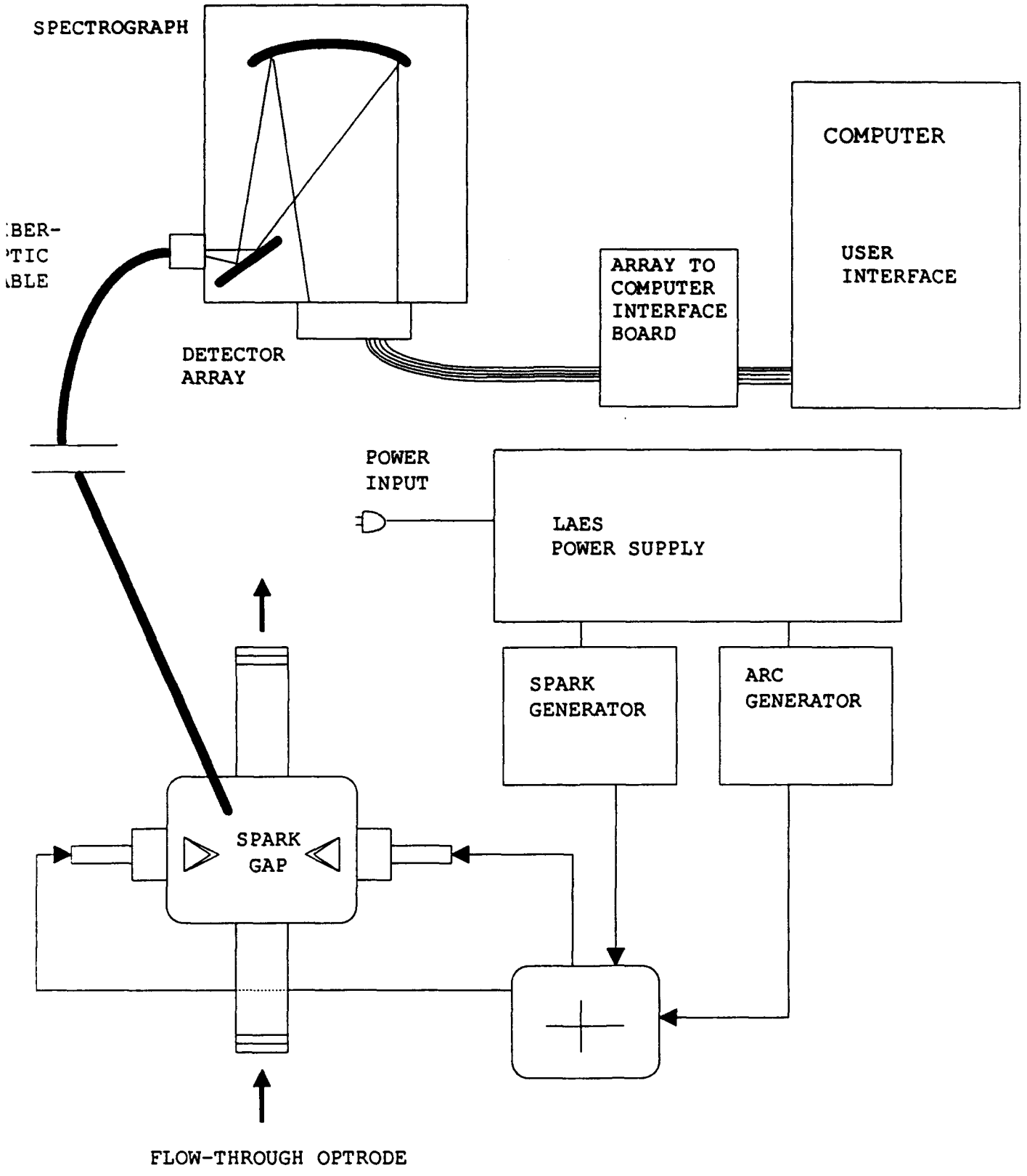

Figure 4-1. Liquid Atomic Emission Spectrometer

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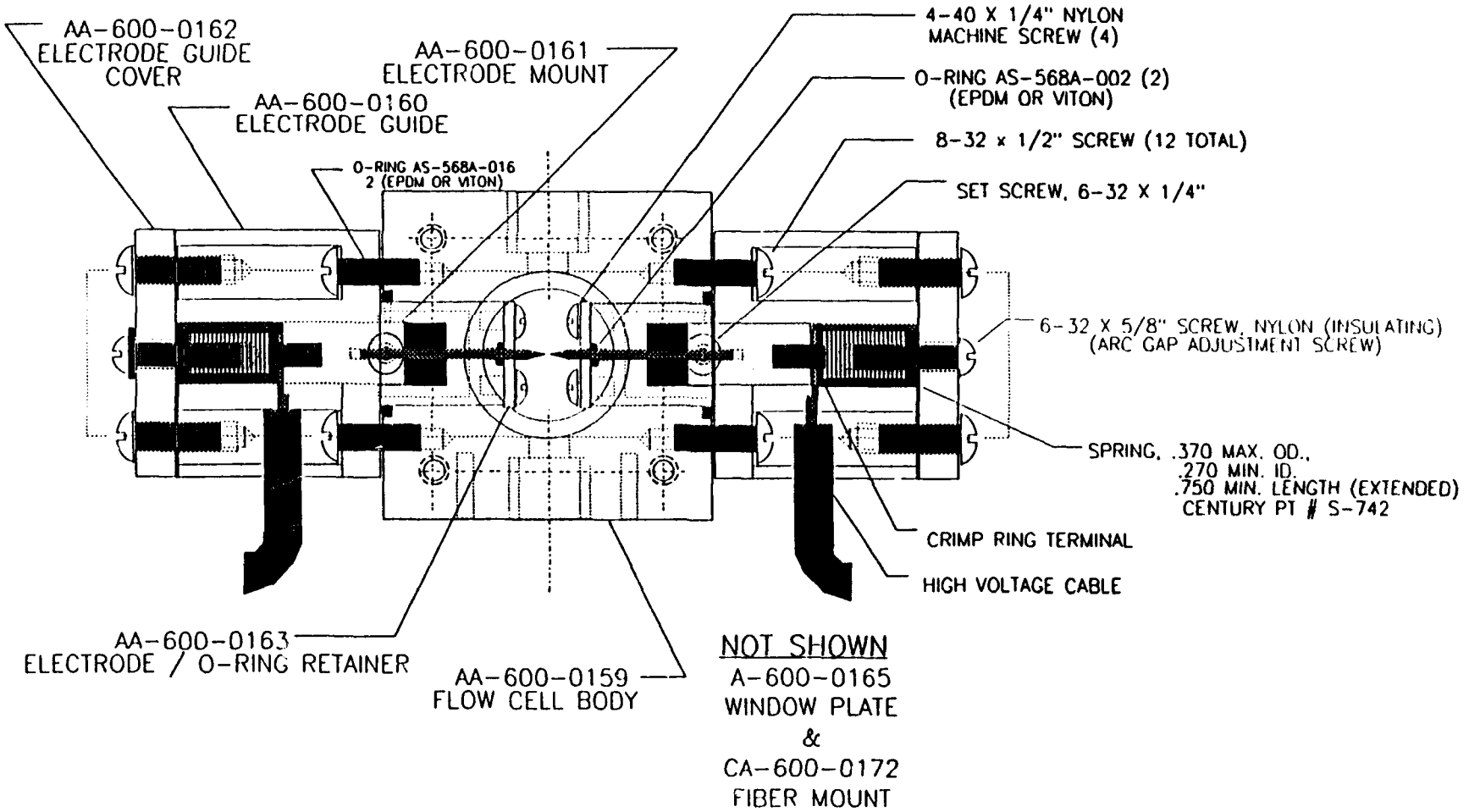

Biotronics Technologies, Inc.

Figure 4-2. LAES Flow Cell

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produces an emission spectrum characteristic of the components in solution. The light from each flow cell is collected by a fiber-optic cable and transmitted to a spectrograph with a fixed holographic grating and linear photodiode array. Figure 4-3 shows the relationships between the major components of the NASA HAES system.

In addition to being used for Phase II work, the NASA HAES served as a starting point in the design of the OHAES system. As described earlier in Section 3, a myriad of improvements and modifications were made to the original HAES system design during the design phase of the project in order to meet the project objectives.

4.4 Oceanographic Hybrid Absorption/Emission Spectrometer (OHAES)

The OHAES system is a hybrid spectrometer capable of collecting and analyzing both absorption and liquid atomic emission spectra. The absorption half of the instrument is based on light from a xenon flash lamp transmitted through a bundled, bifurcated fiber optic cable to two separate flow cells. These flow cells contain spacers so that their path lengths may be varied and different path lengths may be studied simultaneously. The liquid atomic emission portion of the OHAES is based on a high voltage power supply that provides an arc/spark excitation to submerged 18 kt gold electrodes in the emission flow cell. The atomic emission spectra are collected by a fiber-optic cable and passed to fiber-optic cables similar to those that collect light from the two absorption flow cells. Light from the three flow cells is then transmitted to a spectrograph with a fixed holographic grating and detected by a linear photodiode array. Figure 4-4 shows a sketch of the OHAES system layout. Figure 4-5 depicts the relationship between the major components of the OHAES system. For a detailed system specification and additional diagrams, see Appendix A, OHAES System Specifications.

The OHAES was originally planned to be a submersible (towable) optograph with an on-board analyzer. After modifying the contract to take advantage of the sample flow provided by the test vessel, the basic concept of separating the components of the optograph and analyzer still remained in the OHAES design plan. This way it will be easier to redesign the OHAES as a future towable/submersible model.

The OHAES is packaged completely within the confines of a standard 19-inch rack. The top part of the rack contains the analyzer: a 386 MHz computer with coprocessor and a specialty analog to digital communication board. Monitor and keyboard are also part of the analyzer's components. The optograph includes all the hardware necessary to collect the sample's absorption or emission spectra. This includes the flow cells, the spectrograph and photodiode array, the flash lamp (absorbance light source), and the emission power supply (excitation source). In addition, power supplies, junction boards, temperature control, water valves, and filter provisions are required to operate and stabilize the optograph. All the components of the optograph are mounted on one shelf using both the top and bottom of the shelf. The spectrograph and array, flash lamp, temperature controller, and junction boards are on the top of the shelf, while the flow cells, emission power supply, valves, and filter are suspended from the bottom of the shelf. The entire shelf may be extended from the instrument rack for maintenance, and if necessary, the entire shelf may be removed from the rack. In addition, the emission power supply, which is a large and heavy part of the optograph, was mounted to allow easy removal from the optograph shelf if required for maintenance.

There are a total of three flow cells in the OHAES system. Two are for absorbance studies and one is for emission studies. The two absorbance flow cells are specially designed to allow for simple removal and cleaning. This was a new improvement to the OHAES system. In addition, the absorbance flow cells were designed to be used with various flow cell path lengths. Currently installed and calibrated path lengths are 25 mm and 100 mm path lengths. Additional spacers to create other path lengths are available from Biotronics Technologies. The emission flow cell is

HYBRID ABSORPTION/EMISSION SPECTROMETER (MAJOR COMPONENTS)

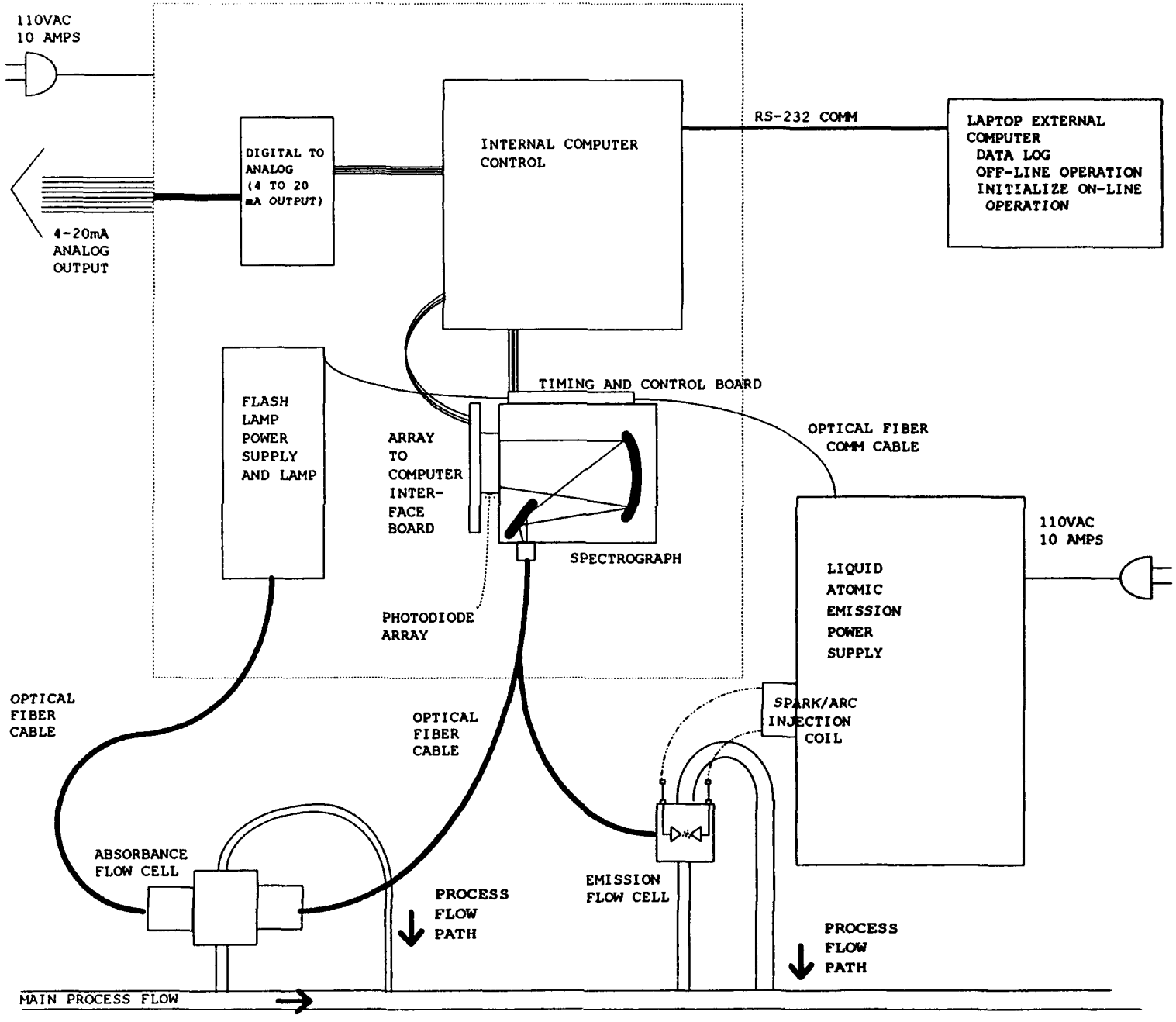

Figure 4-3.

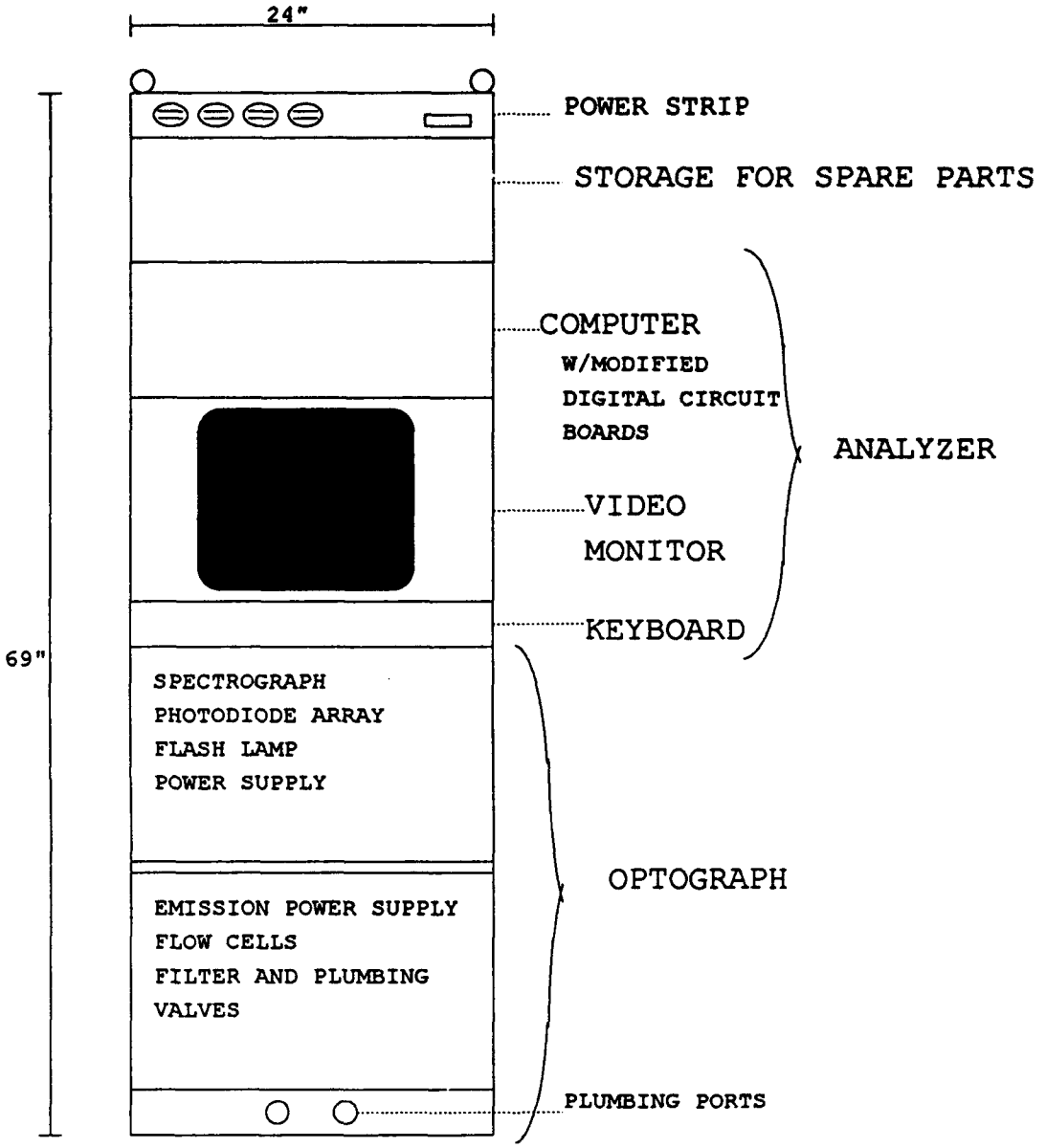

FIGURE 4-4. OHAES SYSTEM LAYOUT

WEIGHT: APPROXIMATELY 425 lbs.

DIMENSIONS: 24"x 33"x 71" (INCLUDING EYE-RINGS)

OCEANOGRAPHIC
HYBRID ABSORPTION/EMISSION SPECTROMETER
OHAES
MAJOR COMPONENTS

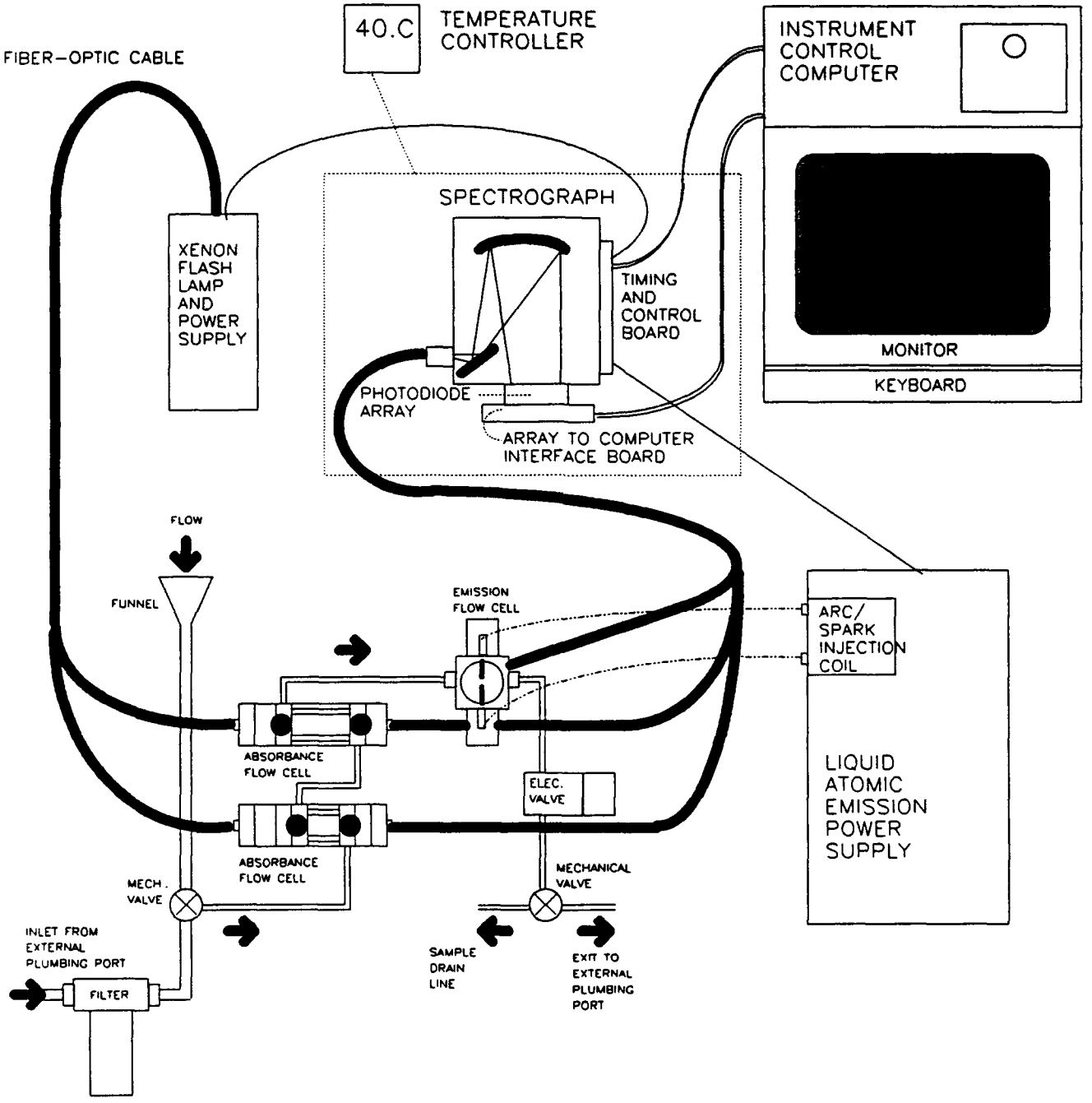

Figure 4-5.

designed with a window so the operator may view the excitation and qualitatively evaluate the emission spectra. In addition, the condition and position of the emission electrodes may be observed through the window. Because of wear of the emission electrodes and the requirement to regularly re-adjust their relative position within the flow cell, this viewing capability saves significant time. Note, the window is made of plastic to shield against ultraviolet radiation.

Additional safety measures have been incorporated into the OHAES instrumentation package. The emission power supply has an interlock with the shelf that will not allow the power supply to initiate an excitation if the shelf is pulled out from the rack. NOTE: this safety feature may be overridden by a trained operator if required for maintenance. However, it should not normally be used and should never be used by anyone not thoroughly trained on the hazards associated with the high voltage emission system.

In addition to the shelf interlock safety measure, ultraviolet shields have been installed to block ultraviolet light from the xenon flash lamp from harming the operator. For detailed safety information, see the OHAES Operator's Manual provided with the system. In addition, the manual has detailed operating and maintenance instructions.

4.5 OHAES Instrument Control Program

All software used to operate the OHAES system was written by Biotronics Technologies. The instrument control software is based on pull-down menus that may be accessed by mouse or keyboard. The OHAES Operator's Manual has detailed explanations of all menu items as well as specific procedures for running instrument standards, collecting spectra from grab samples, operating continuously on-line, and calibrating the instrument. A brief overview of the instrument control program is provided below.

The OHAES control program is titled "OCP.EXE." When the OCP program is initiated a screen appears with a menu line across the top of the screen, instrument status information along the right side of the screen and function key control information along the bottom. The seven menu choices in the OCP program are the following:

FILE EDIT SEARCH CONFIG OPERATE SCAN CALIBRATE

The **FILE**, **EDIT**, and **SEARCH** menus are used to edit ASCII files. The **CONFIG** menu is used to set up the configuration files for individual data scans and continuous on-line runs. In addition, some data file output names are specified within this menu. The **OPERATE** menu may be used to toggle the valve, heater or shutters; to view the current concentration or error file; to reset file counters; and to initiate continuous, on-line operation. The **SCAN** menu is used for off-line scanning. Within this menu, the individual raw data files are named and saved. The **CALIBRATE** menu is used to enter concentrations of known solutions. Refer to the operator's manual for more detailed information on all OHAES software.

5. ANALYTICAL METHODS

Integral to OHAES performance are the analytical methods that convert the raw spectral data into predictions of analyte concentrations. The raw binary data are analyzed by first pre-processing the spectra to put them in a useable form for final analysis. The first section describes the pre-processing required. After pre-processing the binary data, the data files are analyzed using a variety of mathematical algorithms. For absorption spectra, the primary method of solving for the analyte concentrations is principal components analysis. For emission spectra, multiple-variable stepwise regression produced the best results. Additional studies included using neural networks and genetic algorithms. The following sections expand on the mathematical techniques used to move from raw spectral data to analyte concentrations.

5.1 Binary Data Pre-Processing

During a data collection run, raw spectral data are gathered and stored as binary data files. Each binary file actually contains multiple scans of the sample. Normally for absorption studies, 25 scans are taken. For emission runs, 10 scans are taken. In fact, each scan cycle consists of a "light" scan and a "dark" scan. The dark scan is taken when the flash lamp or emission power supply is off. This dark scan is then subtracted from the light scan. By subtracting the dark reading from the reading taken when light is absorbed or emitted by the sample, light leakage and instrument instability should be accounted for. Next, the multiple subtracted scans are averaged for each run to reduce variation. The binary files are reformatted into "JCamp" data files that are standard ASCII files with header information followed by wavelength and light intensity readings.

For absorption, there is very little variance between the multiple scans; however, this pre-processing technique still reduces variation and improves final results. On the other hand, there is significant variation between emission scans because it is difficult to control the energy input to the emission electrodes and therefore it is difficult to stabilize the emission output. A variety of methods were studied during the course of this project to reduce the scan-to-scan variation. Some hardware changes were made as well as changes in electrical instrument settings. In addition, a variety of mathematical filtering techniques were studied to determine whether the use of medians, standard deviations or total light produced could be indicators of which scans to include and how to complete pre-processing of the final data file. Despite experimenting with a variety of complicated and detailed techniques, the optimal method to reduce the scan-to-scan emission variation was to simply average the scans. After averaging, the emission spectra were normalized. Details on that procedure follow in Section 5.3.

5.2 Analysis of Absorption Spectra

For absorption spectra, absorbance is calculated from the averaged JCamp data file, using the distilled water instrument standard and the equation discussed in Section 2.1:

$$A = -\log (I_o/I_i)$$

Knowing the absorbance, the Beer-Lambert Law (see Section 2.1) is applied to find the concentration:

$$A = abc$$

The above equation applies to a single absorbing component in a 100% transmitting (ideal) background. In a multicomponent, real solution, the Beer-Lambert Law is expanded to a set of simultaneous equations with each equation representing a single wavelength:

$$A_1 = k_{11}c_1 + K_{12}c_2 + \ldots + k_{1n}c_n$$

$$A_2 = k_{21}c_1 + k_{22}c_2 + \ldots + k_{2n}c_n$$

$$\vdots$$

$$A_m = K_{m1}c_1 + k_{m2}c_2 + \ldots + k_{mn}c_n$$

or

$$A = KC \text{ (matrix form)}$$

where

k = ab (a constant for a given path length);
n = number of analytes; and
m = number of wavelengths.

In a multicomponent solution in which all of the constituents are known, the concentration vector (C) may be determined algebraically as the solution of the above equation set. This approach has been designated the K-matrix method. This problem is usually solved by the classical least square method. Unfortunately, the existence of unknown components in the liquid being analyzed and non-linear variations of the Beer-Lambert Law make such a solution quite inaccurate in many applications.

To avoid the difficulties presented by unknown components and non-linear variations, the multiple-component Beer-Lambert Law function is usually reformulated in a P-matrix format:

$$c_1 = p_{11}A_1 + p_{12}A_2 + \ldots$$

$$c_2 = p_{21}A_1 + p_{22}A_2 + \ldots$$

$$\vdots$$

$$c_n = p_{n1}A_1 + p_{n2}A_2 + \ldots$$

or

$$C = PA \text{ (matrix form)}$$

where

$$P = K^{-1}.$$

This inverse method has the advantage that it, as opposed to the K-matrix approach, does not require a knowledge of all the absorbing components in solution. The P-matrix approach also allows for the use of a multitude of analytical techniques. These methods include principal components analysis, multiple-variable stepwise regression analysis, discriminant analysis, and neural network analysis. For nitrate, nitrite, ammonia, and copper absorption data, best results have been produced

with principal components analysis of the absorption spectra. For iron, which was also studied via absorption spectroscopy, good results were achieved with principal components analysis only when studied in isolation. Analysis of multicomponent solutions with relatively high concentrations of nitrate (which absorbs at close to the same wavelengths as iron), showed the best results with multiple regression analysis. Details of regression studies are included in Section 5.3

5.2.1 Principal components analysis

Principal components analysis is also called eigenvector analysis, eigenvector decomposition or Karhunen-Loewe expansion[6]. Principal components analysis involves the rotation of an original data vector X to form a new data vector X'

$$X' = AX$$

where

A = an m x m matrix; and
m = number of elements in the original vector.

The A matrix is derived from the covariance matrix or the correlation matrix of a set of representative samples from the population of interest (the "learning set"). The horizontal vectors of the A matrix are called eigenvectors. This matrix is used to transform the original spectral data vector (X) into a transformed vector of independent, uncorrelated variables. The concept of independence is critical to this method of analysis because the intent is to remove the effects of interfering chemical analytes.

Unfortunately, principal components analysis does not provide uncorrelated variables specific to particular analytes. Rather, it generates uncorrelated variables of decreasing levels of variance that correlate to varying degrees with a number of different analytes. Biotronics Technologies has developed a new technique called rotated principal components that produces a single, revised principal component that more completely "explains" a specific analyte[17]. The method of rotating a principal component pair involves converting two principal components into a single principal component with all the information of the original principal component pair. By following an iterative algorithm, a specific rotated principal component will produce a single explanatory variable for each analyte. This single variable allows for simple calibration and estimation of the chemical concentrations of that analyte. More importantly, this variable is independent of the absorbance changes produced by other analyte concentrations.

5.2.2 Pattern Recognition Approach

To evaluate the success of an analytical technique, a two-step pattern recognition approach is used that consists of a learning phase and a test phase. During the learning phase, a set of samples of known composition and concentration is studied using the selected analytical technique and other specified parameters (for example, specific wavelengths). From this study, a specific calibration algorithm is determined. This algorithm is then applied to a test set. Note, this test set is totally separate and not taken from the learning set. Then, the learning set calibration algorithm is applied to the test set spectra to predict concentrations. This technique of using a learning set and test set to evaluate the calibration algorithms was applied to all analytes for both the absorption spectral analysis (described above) and the emission spectral analysis described in the next section.

5.3 Analysis of Emission Spectra

The intensity of light emitted by an element is directly related to the concentration of that analyte[22]. However, while the wavelengths that are emitted by each element are unique, the nature of the optics of the OHAES do not allow resolution of all the emitted light down to the precision necessary to see discrete lines for each element. Instead, there is overlap of the emission among the various elements. Through mathematical analysis, given a large enough set of calibration samples and the appropriate wavelengths, a reasonable estimate of analyte concentration should result.

Because of variations in the control of energy input to the emission system, as well as interference among elements that emit at nearby wavelengths, the analysis of the liquid atomic emission spectra is complex. Literature on atomic emission suggests the use of a reference analyte of a known concentration to help normalize the emission spectra because control of the energy going into the system and the resulting light emitted is very difficult[22]. For the OHAES system, sodium was studied as a possible reference analyte for the emission spectra because its concentration could be estimated, and even quite accurately predicted if salinity data were available. However, experiments comparing the use of sodium for a reference to alternate methods of normalizing the data, showed the two techniques to have very similar error in predicting the test set concentrations. Because these experiments were based on samples with known sodium concentrations, the addition of error into the results by using an estimated or calculated sodium concentration was perceived as being worse than using one of the alternate methods.

The optimal alternate method was selected for normalizing the emission spectra. This method begins with the calculation of the total light (or total energy) emitted during a scan by summing the area under the spectra. Then, each wavelength is divided by this total and multiplied by 1000. This normalization technique produced the best results with the least variation when applied to both learning and test sets.

5.3.1 Multiple Variable Stepwise Regression

After the spectra are pre-processed by normalizing them to the total energy in the scan, the learning sets are analyzed using multiple-variable stepwise regression. During early studies of emission spectra, when experiments were run varying concentrations of only one analyte at a time, a simple single variable (i.e., one wavelength) linear regression produced good correlations and test set predictions of analyte concentrations. However, when concentrations of all analytes vary simultaneously, multiple wavelengths need to be included in the analysis to allow for the possible overlap of interfering spectral lines. Using multiple-variable stepwise regression and studying pertinent ranges of wavelengths for each analyte, the wavelengths for the final calibration are determined. These are then run in another regression to produce the calibration algorithm for the learning set. Then, as explained earlier, this algorithm is applied to a test set to ensure it will be general enough to predict concentrations from spectra not included in the learning set.

In this manner, calibration algorithms are determined for all the analytes being studied. Section 6 summarizes the results for each analyte. Computer programs to perform all preprocessing (e.g., normalizing or computing absorbance) as well as to apply the selected mathematical technique (regression or principal components analysis) have been written by Biotronics Technologies personnel and were included with the software package delivered with the instrument. Detailed instructions on how to run the software are included in the OHAES Operator's Manual.

5.3.2 Neural Networks and Genetic Algorithms

It was apparent from early experience with LAES that a different non-linear form of pattern recognition might be needed for LAES concentration predictions. Multiple variable regression as well as principal components analysis are actually extensions of linear methods based on the principle of superposition. This principle states that the cause-effect results of multiple components in a complex mixture are additive. In other words, these are no second order interactions between components. In atomic emission spectrometry, such interactions are a known physical-chemical phenomenon. For this reason, the development of a non-linear pattern recognition system for the OHAES was studied.

Biotronics Technologies developed a new pattern recognition system that combined the strengths of two currently popular analytical technologies: neural networks and genetic algorithms[16].

Neural networks are a form of mathematical analysis that attempts to simulate the thought process of the human brain. It is currently the most popular form of pattern recognition and is receiving strong financial support form both public and private sources. In the public arena, DARPA, the Navy, and NASA are major supporters of this newly revived technology. Although a wide variety of neural nets have been developed, all are made up of a multitude of processing elements called neurons or nodes. These neurons are structured into vast networks that allow for complex non-linear interactions between the neuron elements.

There are two major weaknesses that limit the use of neural networks:

1. Long training times; and
2. Non-optimal behavior.

Many neural networks, particularly those large in size, often require long training times. Some nets may require days to reach a solution even using a high speed computer. Such learning times may seem only a nuisance, because once the pattern is "learned," the process does not need to be repeated. The second weakness is a bigger problem. Even after a long training time, the neural network may still not achieve the best solution. In the parlance of the field, this result is called getting stuck in a "local" minimum. Because of this, Biotronics Technologies elected to combine neural network technology with another new pattern recognition technique - genetic algorithms.

Genetic algorithms use the technique of human genetics including cell reproduction, merger, and mutation to optimize a pattern recognition system. In combination with a neural network, they not only shorten training times, but also provide optimal parametric solutions. Biotronics Technologies developed software combining these technologies in a software package called NETGEN[16]. During Phase II of this project, extensive analysis of spectral data was completed using NETGEN software. Section 6 summarizes all the analytical results.

6. ANALYTICAL RESULTS

This section summarizes the analytical results for each phase of the project. Each phase built on the results of earlier phases, becoming more complex with each step.

6.1 Phase I - Individual Analyte Analysis

The major objective of the Phase I work activity was to generate basic spectra for all of the analytes of interest in the appropriate concentration ranges using absorption and/or emission spectrometers. The intent was to determine whether it was possible to measure variations in concentration of the individual analytes in isolation via reagentless absorption or emission spectral analysis. Absorption studies were performed on a Perkin Elmer Lambda 9 Spectrophotometer. Emission studies were completed on a prototype Liquid Atomic Emission Spectrometer (LAES). Strong absorption spectra were found for the following analytes:

1. Nitrate
2. Nitrite
3. Ammonia
4. Iron

Figures 6-1 through 6-4 show the absorbance curves for each of the above analytes in distilled water for samples of varying concentration. Analyte concentrations are shown on the plots. Note, the concentrations for nitrate and nitrite are NOT "ppb nitrate as nitrogen" or "ppb nitrite as nitrogen" as is sometimes reported by laboratories. To convert to nitrate as nitrogen (NO3-N) or nitrite as nitrogen (NO2-N) multiply all figures throughout this paper by 0.226 for nitrate or 0.304 for nitrite. Similarly, to convert to ammonia as nitrogen (NH3-N) multiply the figures in this report by 0.823.

Both the nitrate and nitrite curves (Figures 6-1 and 6-2) are strong and track well with increasing concentrations. The ammonia curves (Figure 6-3) look good, but the "shoulder" of the ammonia absorbance peak begins at approximately 220 nm and extends into the vacuum ultraviolet wavelength range. This may be beyond the detectable limit of the planned optical system. The iron absorbance curves (Figure 6-4) correlate with concentration, but the low levels of absorbance (due to the low levels of iron being studied) put the absorbance near the noise level of the instrument. In addition, complex solutions may hide the iron absorption spectra.

The following four analytes had measurable absorbance spectra that did not correlate as well with analyte concentration.

1. Copper
2. Silica (in colloidal suspension)
3. Molybdenum
4. Zinc

Examples of these absorbance curves are shown in Figures 6-5 through 6-8. The copper absorbance curves (Figure 6-5) did not track with increasing concentration, possibly due to the low levels being studied. The silica absorbance curves (Figure 6-6) are basically flat lines relating to the turbidity of the solution rather than to the silica concentration. In real world sample solutions, it may not be possible to separate the absorbance due to silica from other sources of turbidity or from optical fouling. Molybdenum (Figure 6-7) and zinc (Figure 6-8) absorbance spectra are weak and do not correlate with concentration.

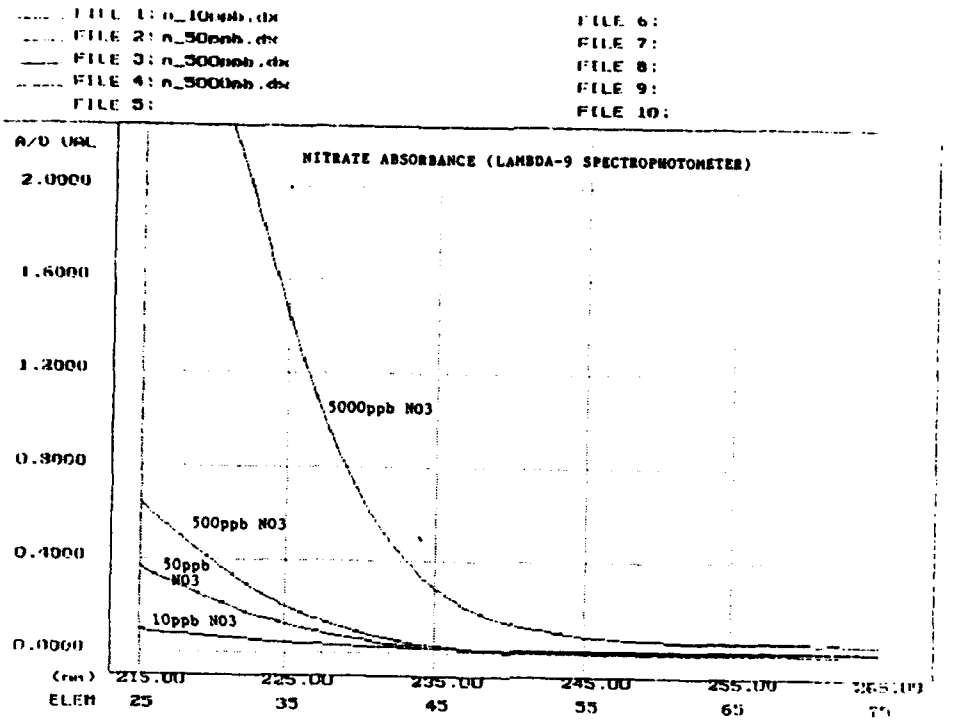

Figure 6-1.

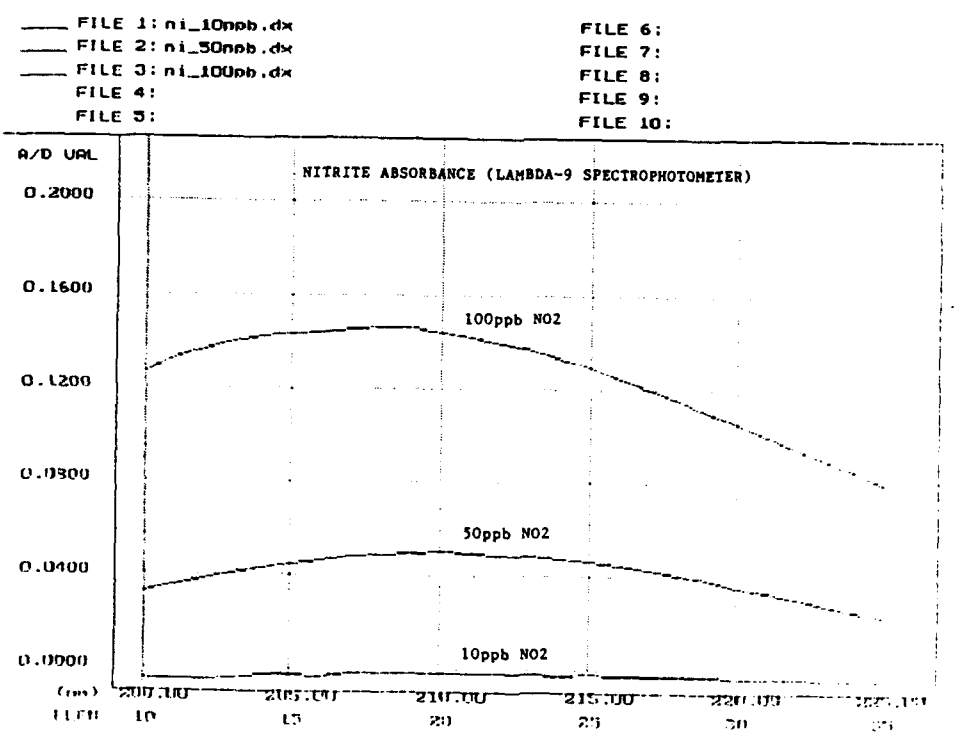

Figure 6-2

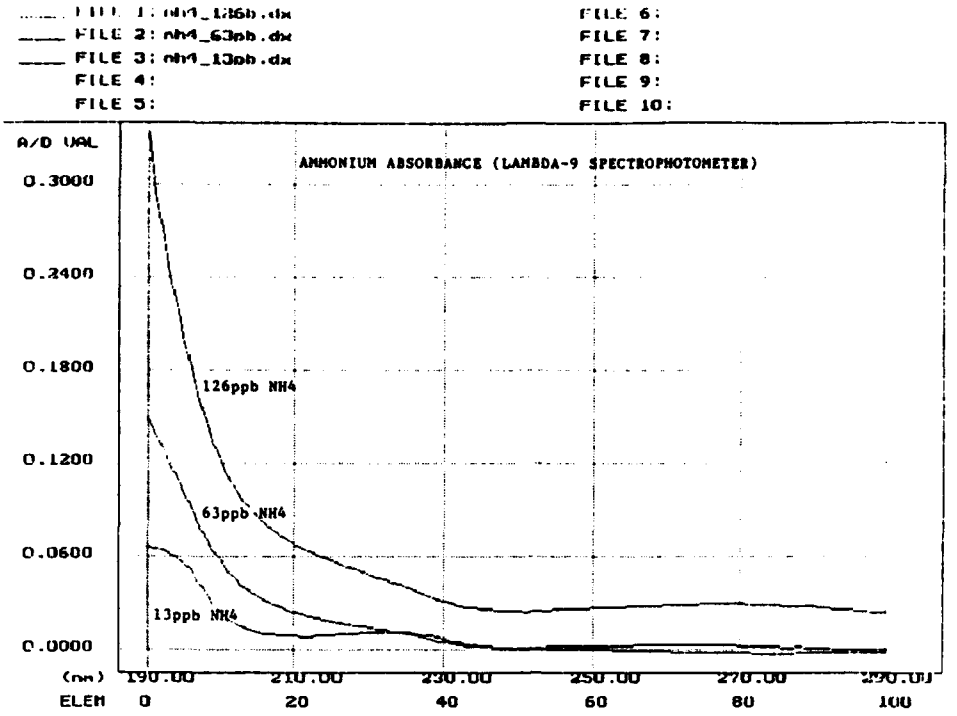

Figure 6-3.

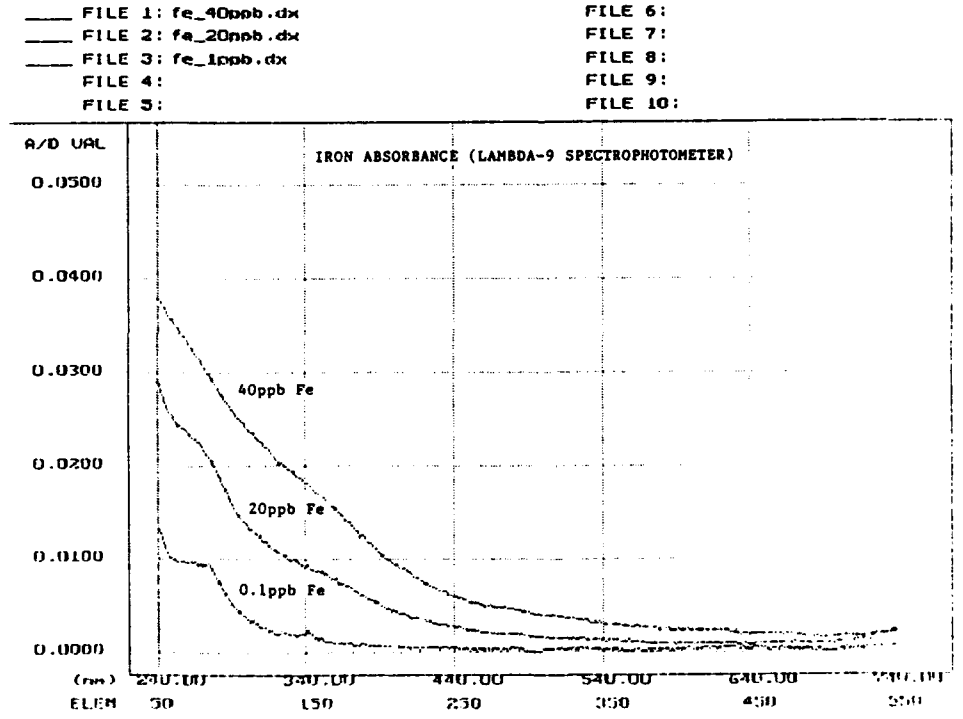

Figure 6-4.

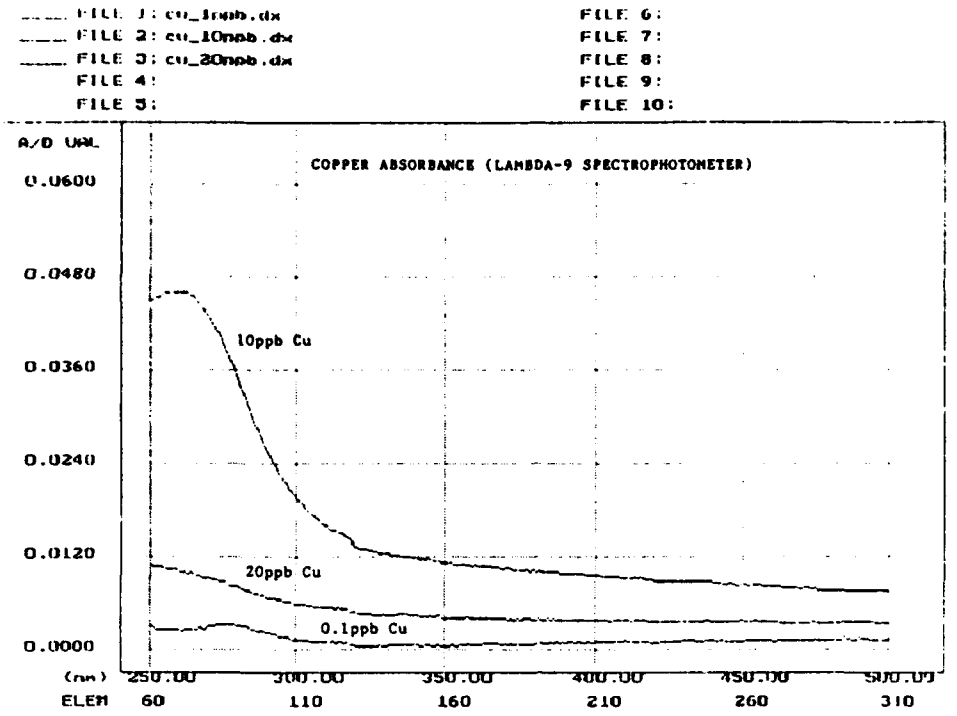

Figure 6-5.

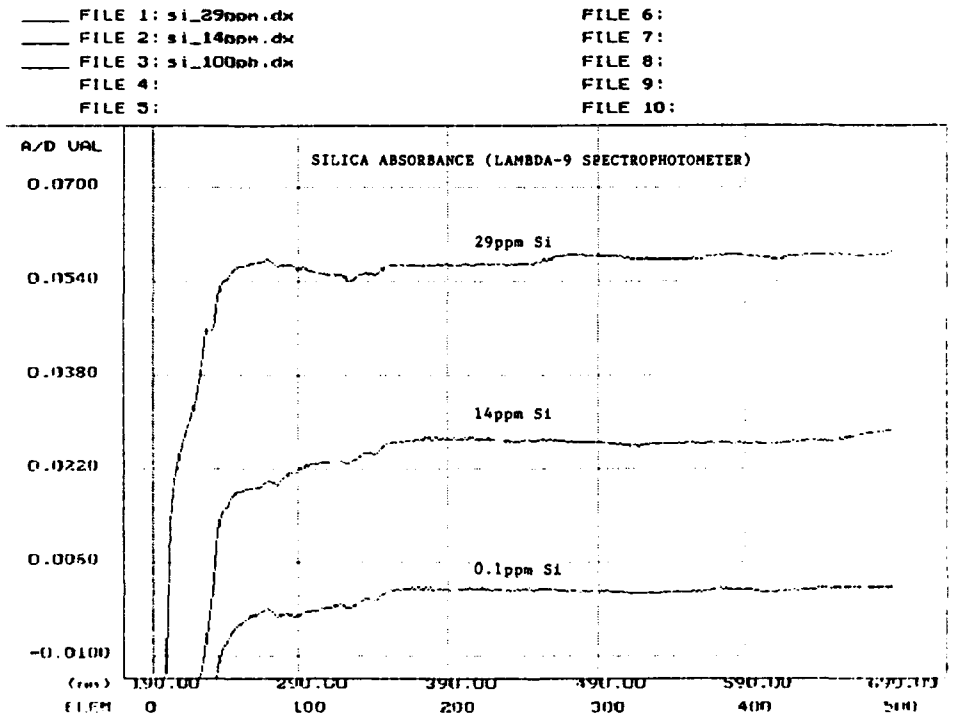

Figure 6-6.

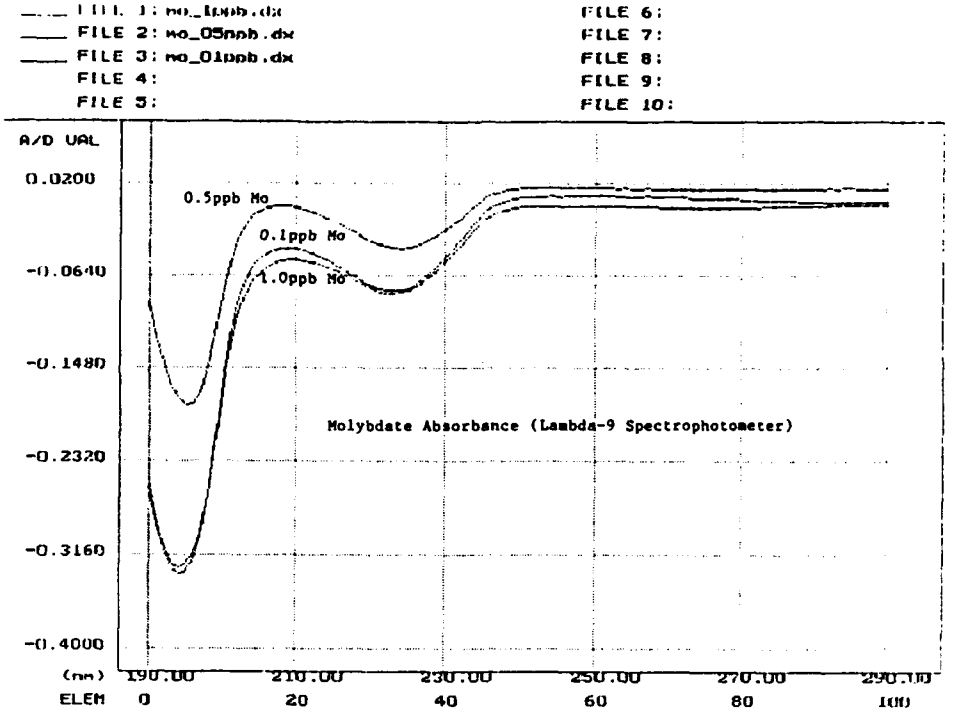

Figure 6-7.

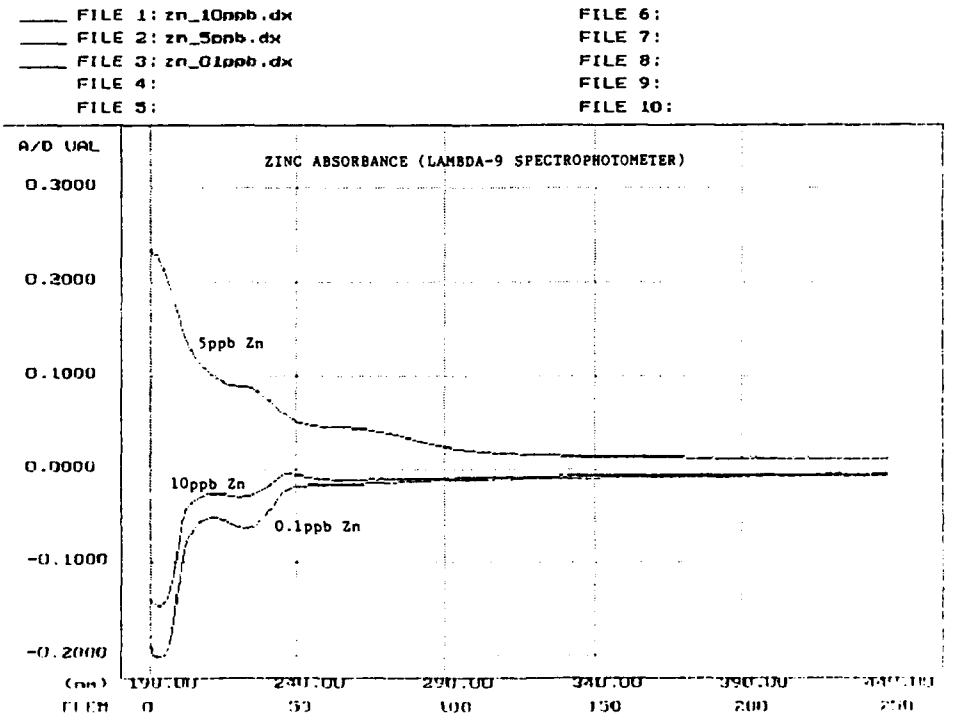

Figure 6-8.

Strong atomic emission spectra that tracked with increasing concentrations were recorded with for the following analytes:

1. Calcium
2. Magnesium
3. Potassium

The emission curves showing the relationship with concentration for each analyte are included as Figures 6-9 through 6-11. The emission spectra have been normalized to total energy in all of these figures.

Phosphate was the only key nutrient analyte that did not have any reagentless spectra (absorption or emission) that would correlate with concentration. Additional work with phosphorous (versus phosphate) in the atomic emission domain was planned for Phase II to determine whether phosphorous emission spectra could be used to predict phosphate concentration.

6.2 Phase II - Multiple Analyte Analysis

Phase II activity built on Phase I activity by studying the same analytes but in multicomponent solutions, not as isolates. For each sample mixture, absorption and emission scans were taken. All analyte concentrations were varied simultaneously in each sample in an Instant Ocean background. Instant Ocean is a commercially available compound that simulates the various salts in typical ocean water. Molybdenum and zinc were not studied during this phase because their concentration in the Instant Ocean compound was greater than the levels desired for study.

After the spectral data were collected, several different pattern recognition techniques were used to find the optimal algorithm for predicting concentrations. A learning set was used to determine the algorithm for each analyte, and a separate test set was then used to evaluate the application of the final algorithm. The results shown in Tables 1 and 2 are based on Biotronics Technologies' NETGEN program, a genetic neural network software package.

Table 1: Absorption Phase II Test Results

ANALYTE	RANGE	AVG. ERROR	AVG. % ERROR	SLOPE	T-VALUE
Nitrate (NO_3)	10-500 ppb	11.3 ppb	9.2%	1.001	13.6
Nitrite (NO_2)	10-50 ppb	1.6 ppb	15.6%	0.542	5.2
Ammonia (NH_4)	12.5-125 ppb	5.1 ppb	18.3%	0.177	2.3
Copper (Cu)	1-20 ppb	0.4 ppb	18.6%	0.132	2.4
Iron (Fe)	12-40 ppb	1.8 ppb	25.7%	0.296	1.78

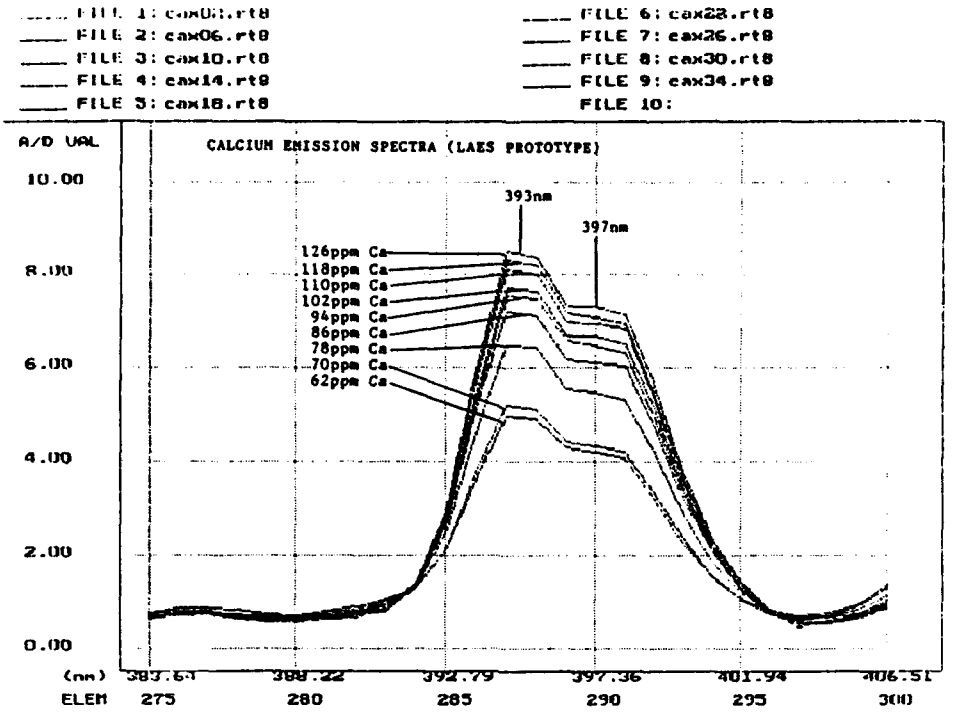

Figure 6-9.

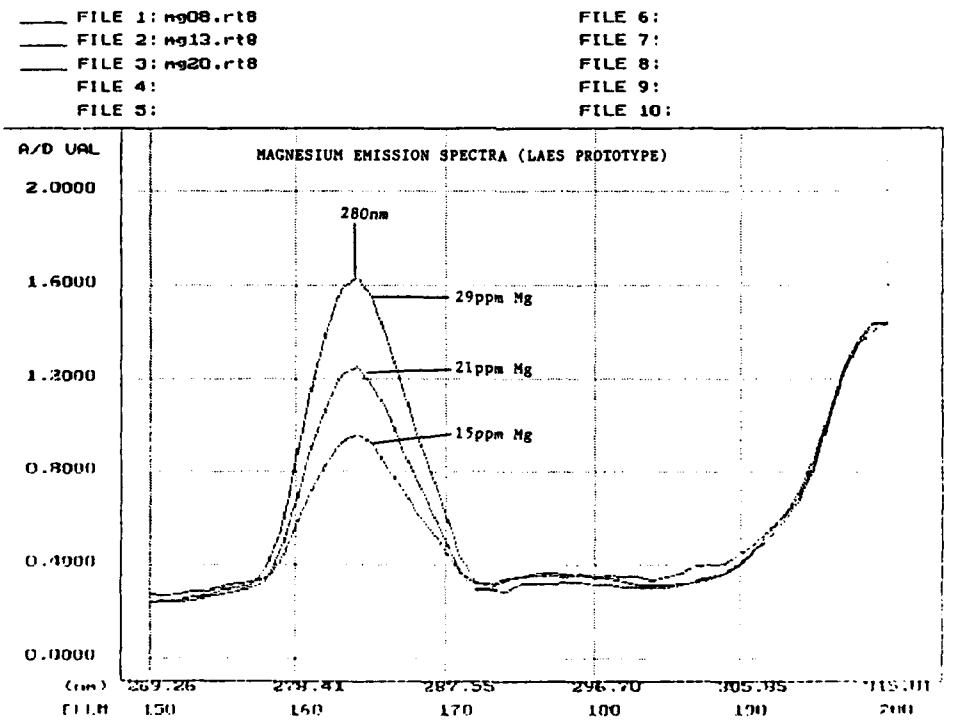

Figure 6-10.

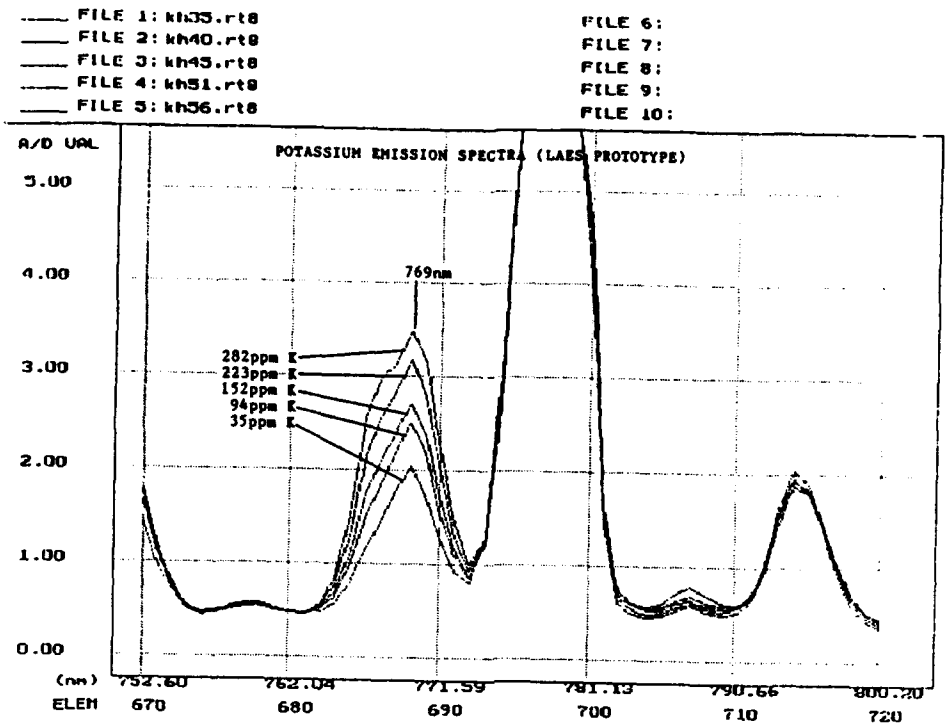
FILE 1: kh35.rt8
FILE 2: kh40.rt8
FILE 3: kh45.rt8
FILE 4: kh51.rt8
FILE 5: kh56.rt8
FILE 6:
FILE 7:
FILE 8:
FILE 9:
FILE 10:
POTASSIUM EMISSION SPECTRA (LAES PROTOTYPE)
A/D VAL
5.00
4.00
3.00
2.00
1.00
0.00
769nm
282ppm K
223ppm K
152ppm K
94ppm K
35ppm K
(nm)
752.60
762.04
771.59
781.13
790.66
800.20
ELEM
670
680
690
700
710
720

Figure 6-11.

Table 2: Emission Phase II Test Results

ANALYTE	RANGE	AVG. ERROR	AVG. % ERROR	SLOPE	T-VALUE
Calcium (Ca)	420-800 ppm	8.3 ppm	8.3%	0.887	8.3
Magnesium (Mg)	1400-2544 ppm	27.5 ppm	9.6%	0.732	27.5
Potassium (K)	360-760 ppm	6.6 ppm	6.6%	0.885	12.1
Silica (SiO_4)	0.3-29 ppm	1.2 ppm	17.4%	0.315	2.17
Phosphate (PO_4)	0-50 ppm	2.5 ppm	19.7%	0.054	0.66

The slope shown in Tables 1 and 2 is the slope of the regression of the actual versus the predicted analyte concentrations. Ideally, the slope should be one and the average error should be low. As the slope moves toward zero, the algorithm is actually predicting a mean rather than tracking with increases and decreases in the actual concentrations. Five analytes from the tables above are identified as strong analytes; their slopes are greater than 0.5 with relatively low average errors.

1. Nitrate
2. Nitrite
3. Calcium
4. Magnesium
5. Potassium

Reagentless spectral determination of concentration for these five strong analytes should be achievable with Biotronics Technologies' OHAES prototype to be designed and built in the next phase. The remaining five analytes (ammonia, copper, iron, silica, and phosphate) need additional work to improve the possibility of reagentless spectral determination of concentration in ocean water.

One means to improve the potential performance of the OHAES for some of these analytes was to re-evaluate the range being studied. A slightly wider range may improve the algorithms' ability to track with changes in concentration and therefore improve (increase) the slope and reduce the average error. In addition to adjusting the ranges being studied due to statistical requirements, Environmental Protection Agency (EPA) data[3,4,5] of analyte concentrations collected over several years in the Chesapeake Bay and marine textbooks[11] suggest changes to be made to the nitrate, nitrite, and phosphate concentration ranges provided in Tables 1 and 2. Finally, because field testing became limited to the Chesapeake Bay (due to time and Naval Academy restrictions) where the expected salinity is approximately one-third that in open ocean waters, the ranges for calcium, magnesium, and potassium (all conservative ions that are directly related to salinity[11]) must be adjusted accordingly. The final ranges to be used in the Phase III design effort take into account the physical and statistical expectations of the Phase IV test environment. These ranges are shown in Table 3.

Table 3: Analyte Ranges and Expected Averages and Maximums

ANALYTE	RANGE	AVERAGE EXPECTED	MAXIMUM EXPECTED
Nitrate	10-5000 ppb	1500 ppb	3500 ppb
Nitrite	10-500 ppb	75 ppb	500 ppb
Ammonia	10-500 ppb	75 ppb	350 ppb
Copper	0-50 ppb	5 ppb	10 ppb
Iron	0-50 ppb	3 ppb	6 ppb
Calcium	50-600 ppm	140 ppm Chesapeake Bay	420 ppm Open Ocean
Magnesium	300-1800 ppm	430 ppm Chesapeake Bay	1290 ppm Open Ocean
Potassium	50-600 ppm	135 ppm Chesapeake Bay	400 ppm Open Ocean
Silica	0-30 ppm	3 ppm	10 ppm
Phosphate	0-500 ppb	25 ppb	120 ppb

Finally, molybdenum and zinc are present only in sub-part per billion concentrations and are expected to be below the levels of detection capable with the current version of liquid atomic emission technology. Therefore, these will not be included until this technology is able to detect such low concentrations.

6.3 Phase III - New System Development and Simulated Field Test (Calibration)

During Phase III of this project, the OHAES was designed, manufactured, and tested in-house at Biotronics Technologies. Details on the OHAES are included in Section 4, Instrumentation, and in Appendix A, OHAES System Specifications. This section covers the analytical results of the simulated field testing.

To simulate a field test at Biotronics Technologies, the OHAES was calibrated with samples that mimicked the field test conditions expected in the Chesapeake Bay. Forty samples were prepared in which the concentration of ten analytes varied randomly in a one-third strength Instant Ocean background. Less than full strength Instant Ocean was used because the average salinity of the Chesapeake Bay is approximately one-third that of open ocean waters. The ten analytes for which calibration algorithms were developed include nitrate, nitrite, ammonia, copper, iron, calcium, magnesium, potassium, silica, and phosphate. The first five analytes were studied with absorption spectroscopy in two flow cells of different path lengths (25 mm and 100 mm). The last five analytes were studied with emission spectroscopy in a separate flow cell.

Each of the samples was run three times. Then the data were divided into two groups, a learning set and a test set. The learning set was used to determine the optimal algorithm for predicting analyte concentration. Then, the test set was used to test that algorithm. A variety of mathematical

techniques and wavelengths were used to determine the optimal algorithm. Table 4 summarizes the results for the learning set (LS) and the test set (TS). The accuracy of the results is evaluated by examining coefficient of determination (R^2), which is the variation as explained by a regression model divided by the total variation of the data. An R^2 of 1 would mean the mathematical model could perfectly fit all data points. In general, the closer to 1, the better the model fits.

Table 4: OHAES Original Calibration Summary (October '93)

ANALYTE	RANGE	LS R^2	TS AVG ERROR	TS SLOPE	TS T-VALUE
Nitrate	10-5000 ppb	0.999	44.6 ppb	1.00	152.6
Nitrite	10-500 ppb	0.924	41.6 ppb	0.88	15.8
Ammonia	10-500 ppb	0.360	95.3 ppb	0.36	4.7
Copper	0-50 ppb	0.620	7.9 ppb	0.62	12.0
Iron	0-50 ppb	0.507	7.3 ppb	0.50	4.3
Calcium	50-600 ppm	0.847	60.1 ppm	0.72	11.5
Magnesium	300-1800 ppm	0.826	186.1 ppm	0.74	10.7
Potassium	50-600 ppm	0.799	65.3 ppm	0.67	13.0
Silica	0-30 ppm	0.436	5.4 ppm	0.52	4.6
Phosphate	0-500 ppb	0.367	91.2 ppm	0.45	5.2

Excellent calibrations were achieved for nitrate and nitrite ($R^2 > 0.9$). Good calibrations were completed for calcium, magnesium, and potassium ($R^2 > 0.75$). The copper and iron calibrations were less robust, but still provide reasonable prediction power ($R^2 > 0.5$). The ammonia, silica, and phosphate calibrations tend to predict the average of the given range. They do not track well with actual concentrations.

Figures 6-12 through 6-31 show the learning and test set regression plots of actual versus predicted concentrations for each analyte. After completion of the in-house (simulated) field test, the OHAES was secured for shipment and sent to the U.S. Naval Academy in Annapolis, Maryland for field testing in the Chesapeake Bay.

6.4 Phase IV - Ocean/Bay Field Test

Phase IV of this project consisted of instrument installation, four cruises to accomplish field testing, and data analysis and evaluation. The OHAES was installed aboard the YP-686, a U.S. Navy yard patrol craft based at the Naval Academy, which is often used by the Naval Academy's Oceanography Department as well as NOAA for ocean and bay research. In fact, the first two test cruises were accomplished in conjunction with Dr. John Foerster's oceanography class cruises on October 28, 1993. The third cruise was completed November 18, 1993. This cruise was a long cruise to allow for maximum sample collection over a widely varying area. A fourth cruise was added on January 28, 1994, to repeat the nitrate studies because of errors in earlier laboratory analyses of nitrate concentrations.

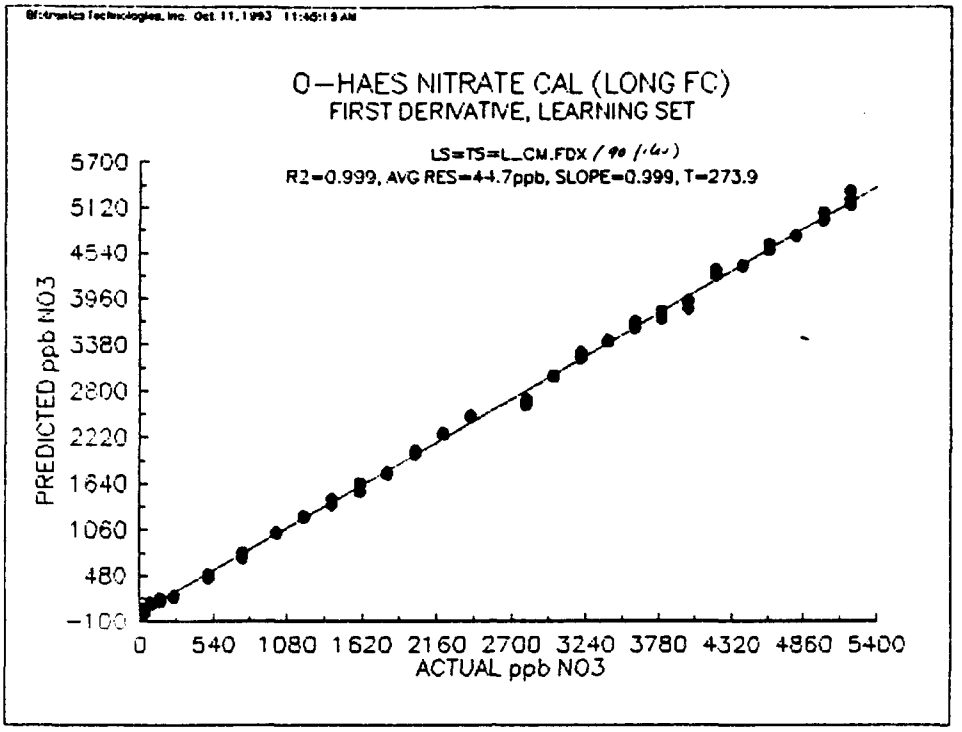

Figure 6-12.

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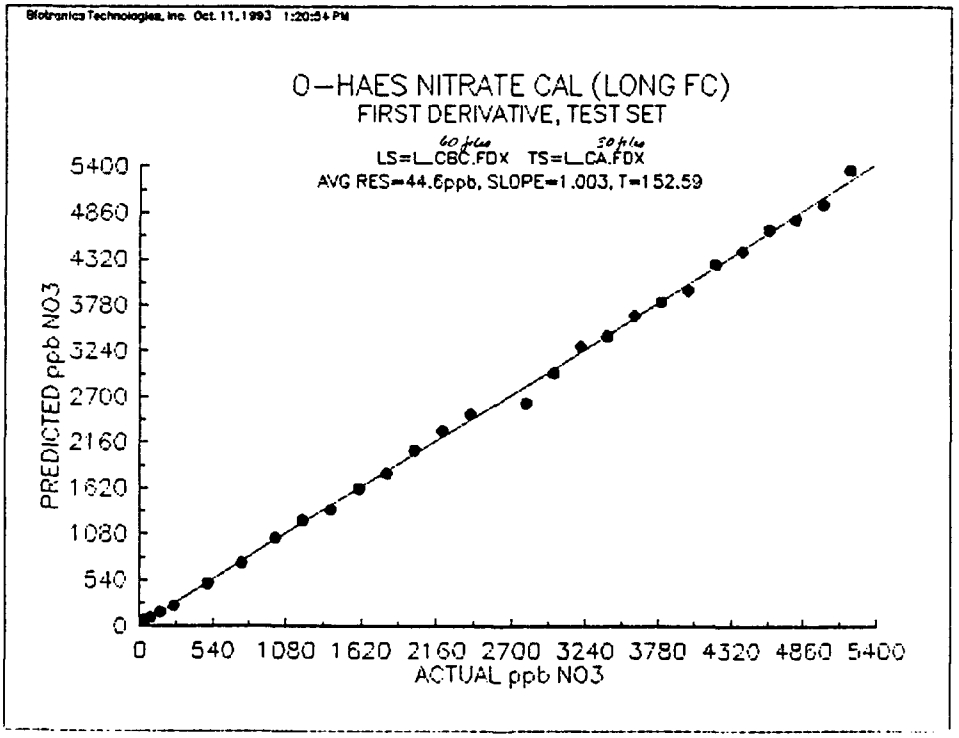

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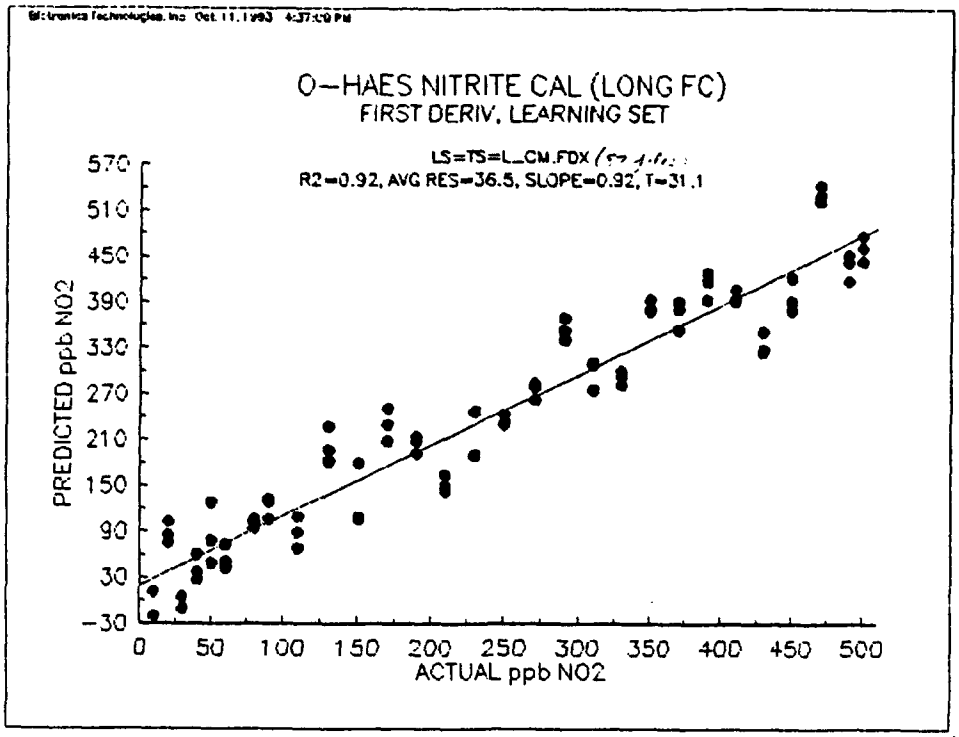

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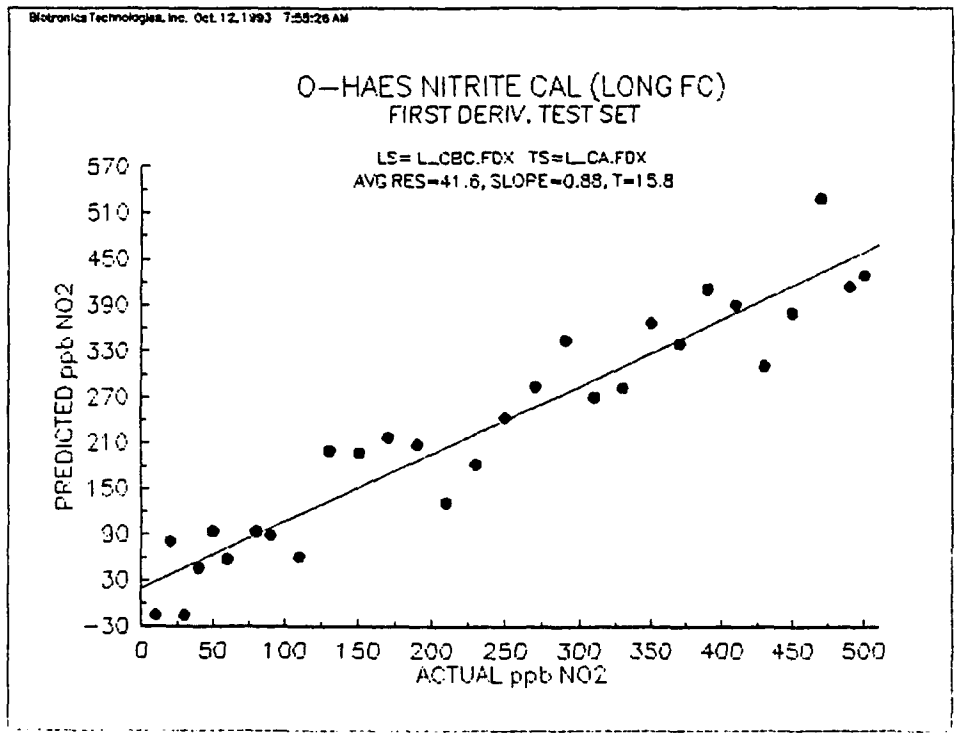

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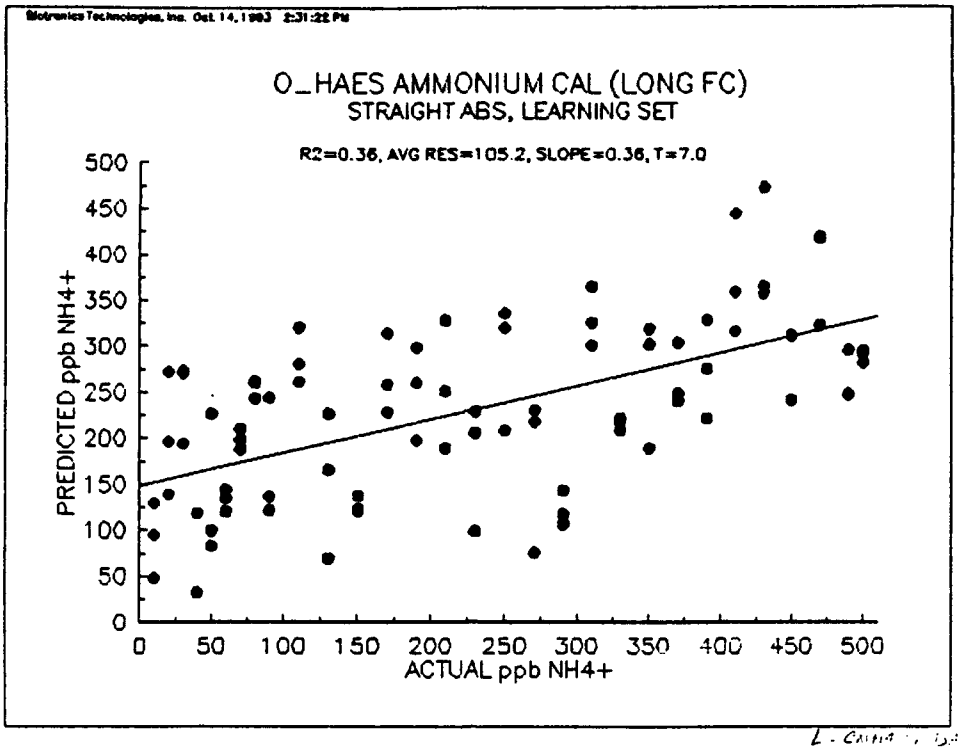

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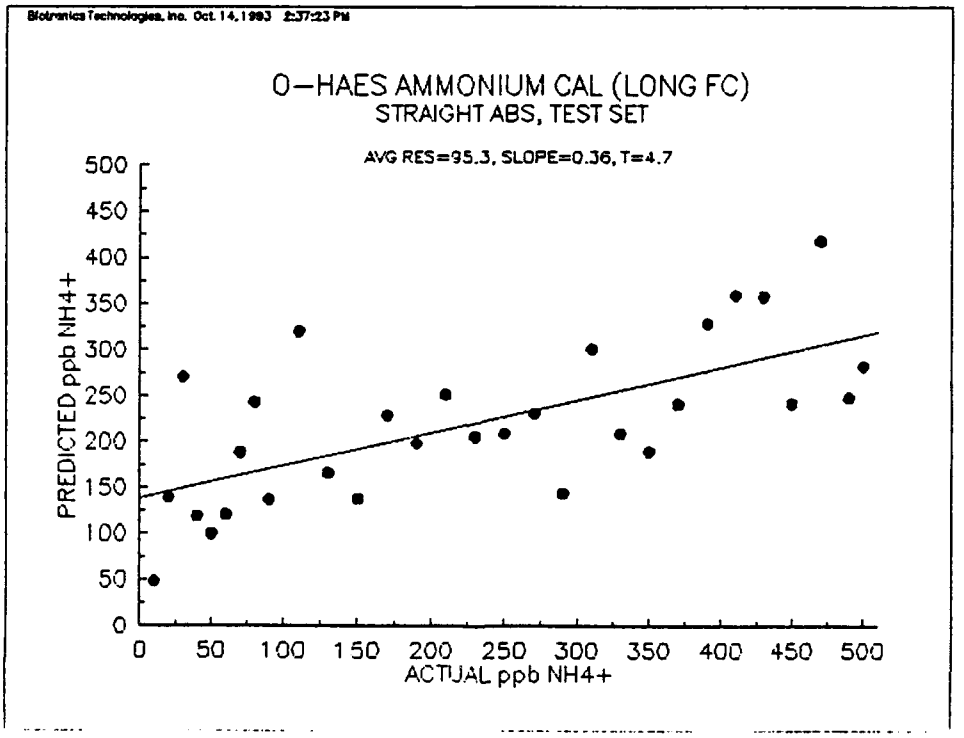

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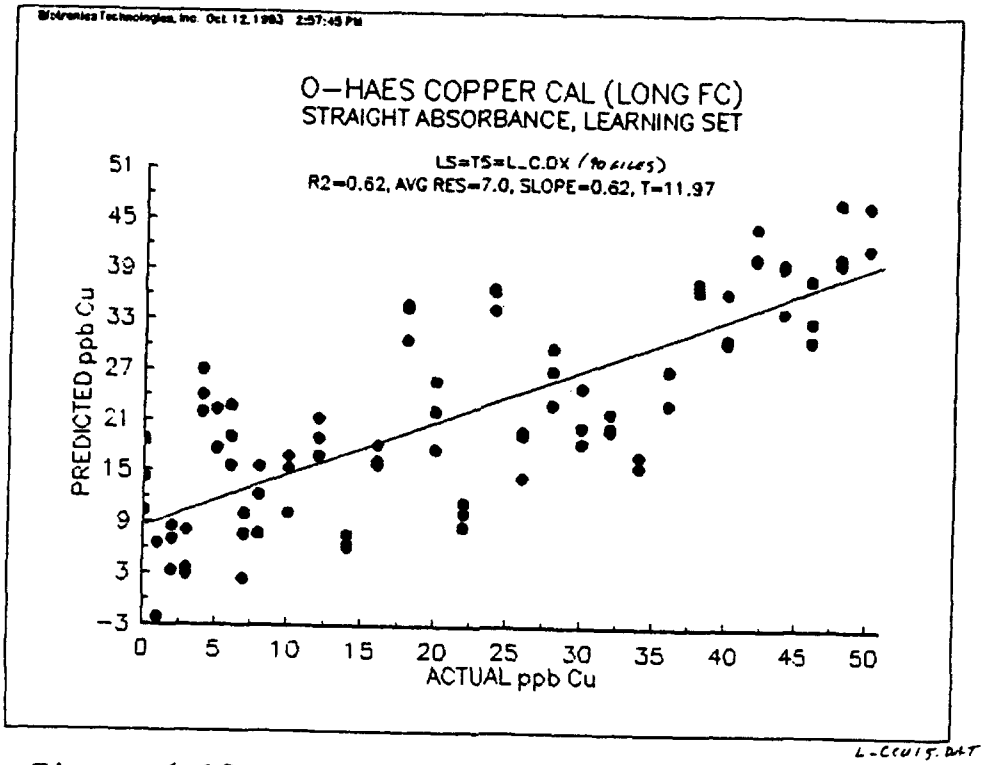

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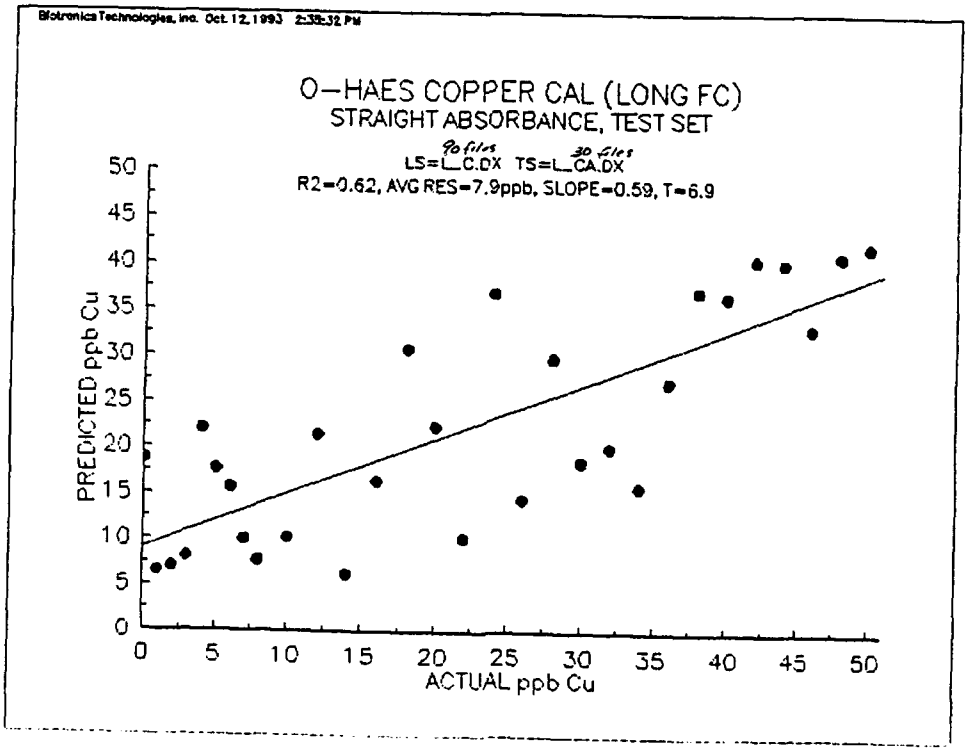

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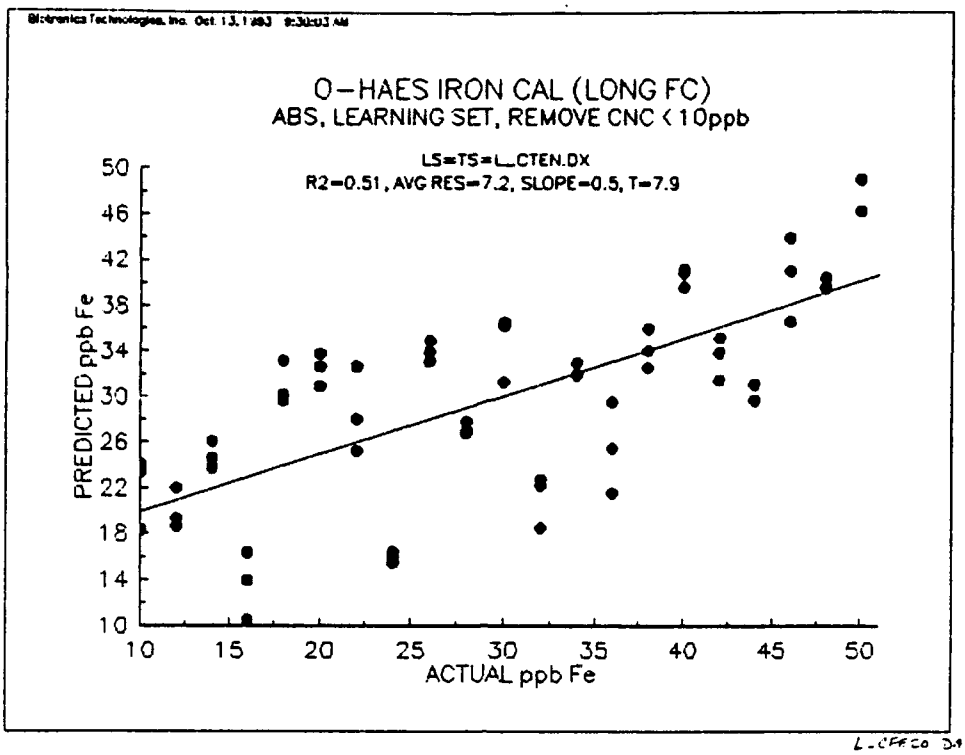

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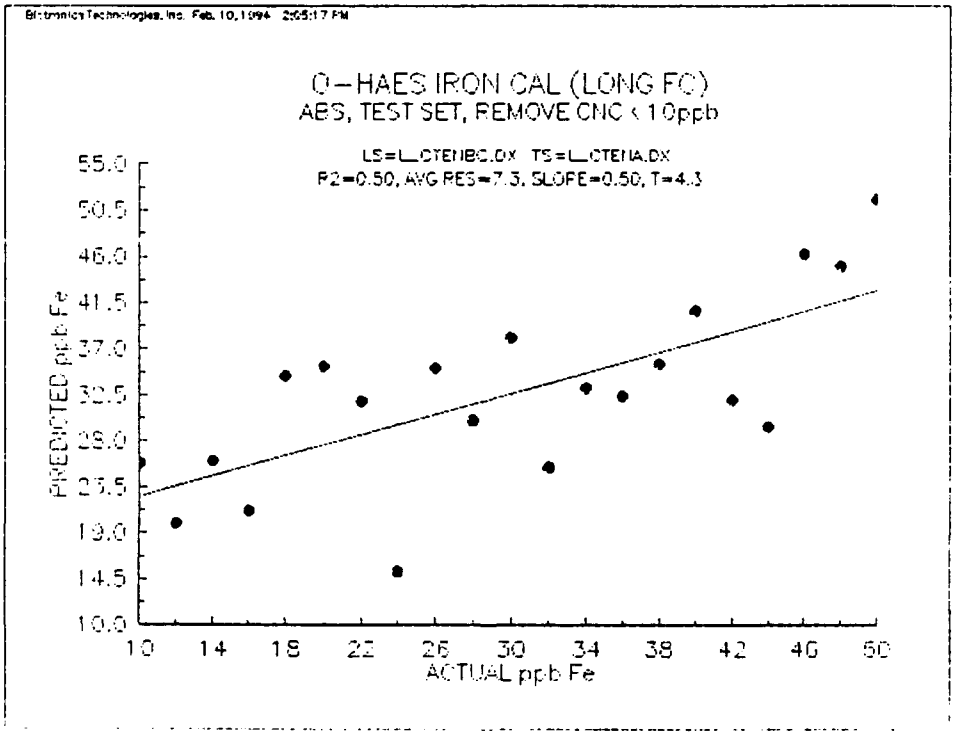

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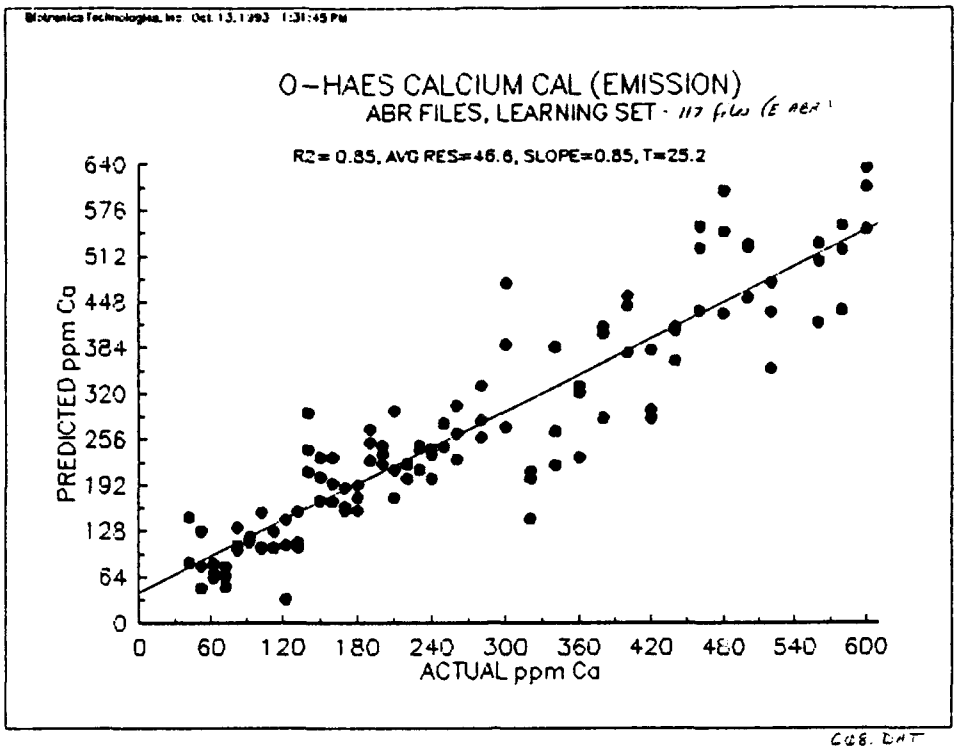

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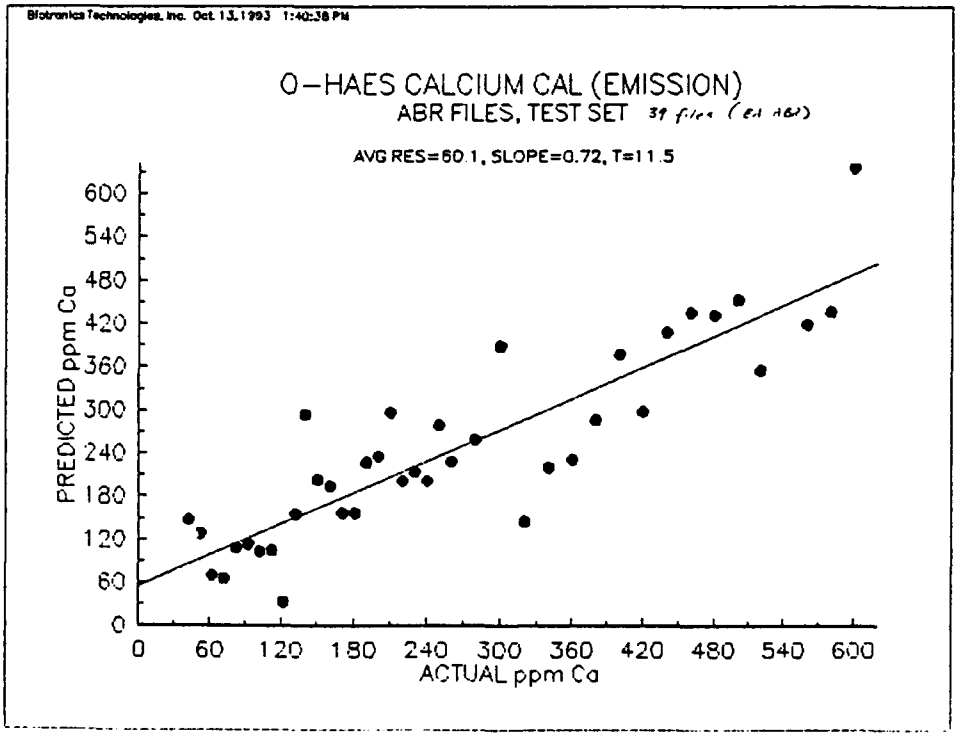

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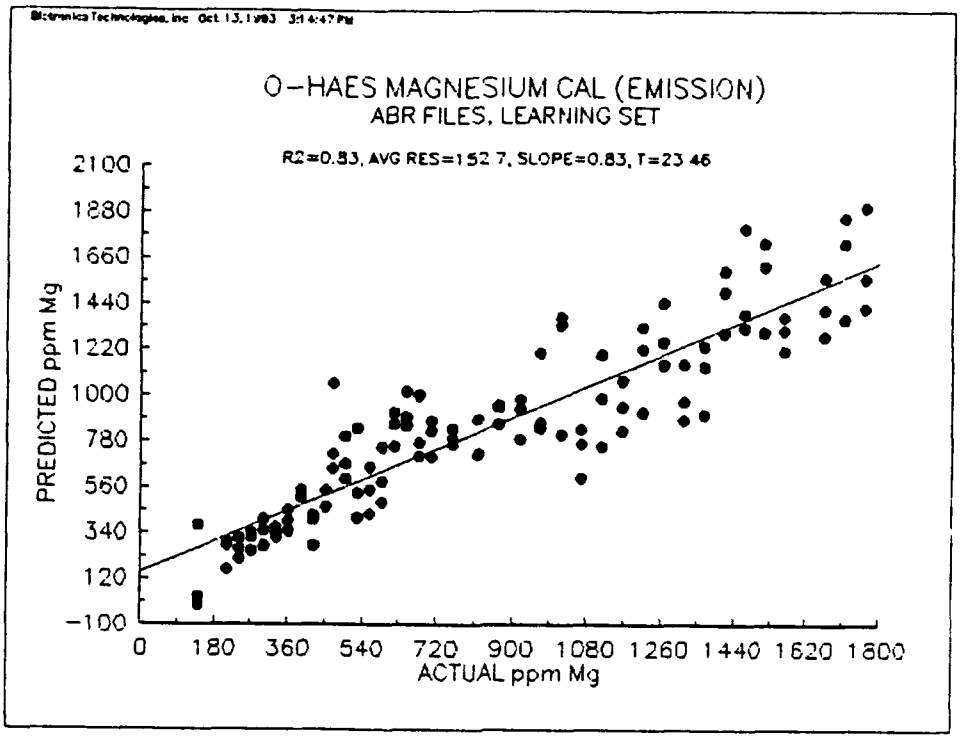

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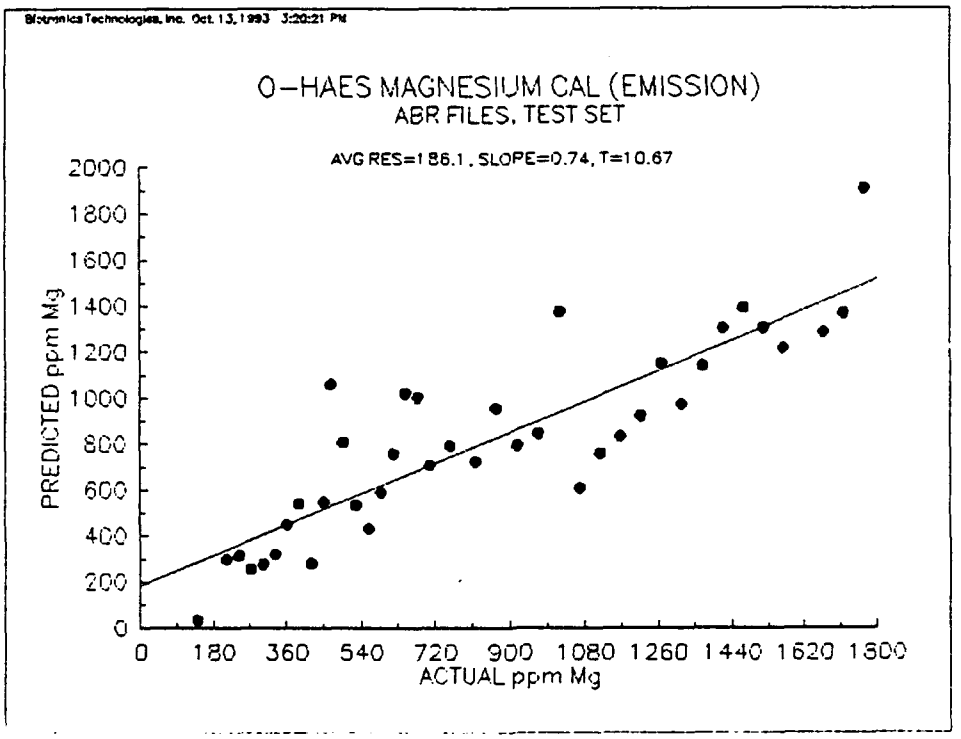

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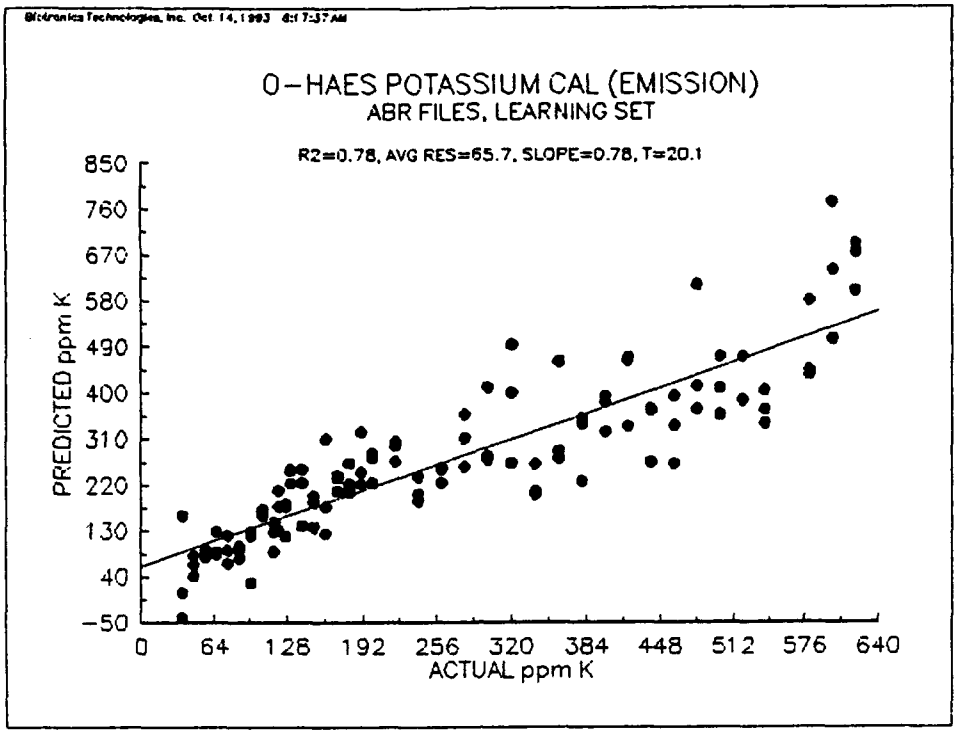

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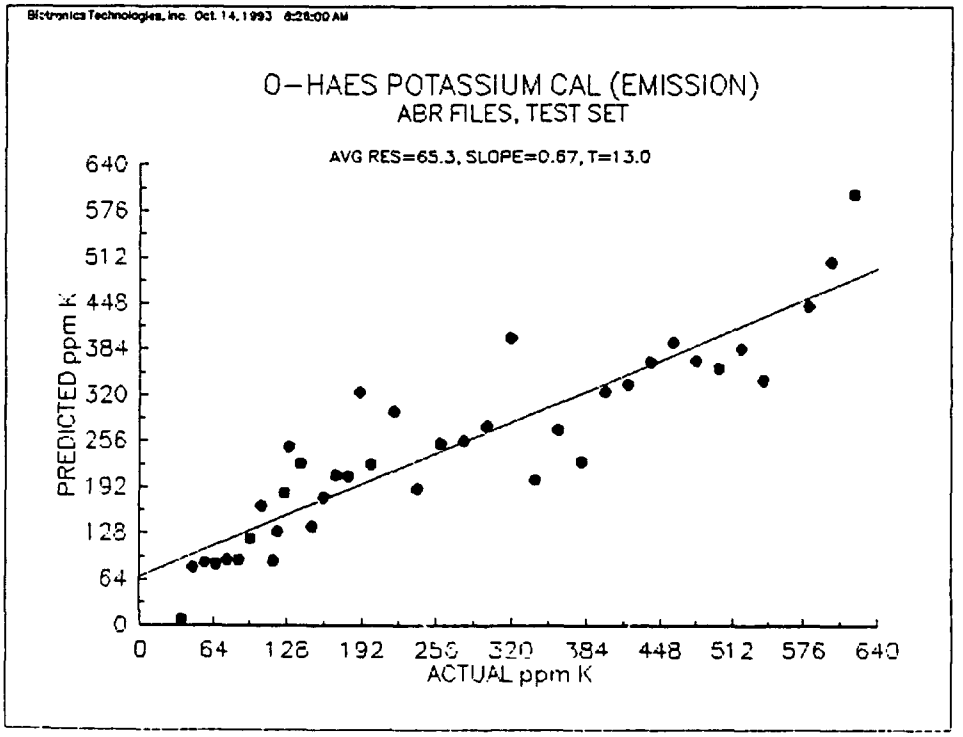

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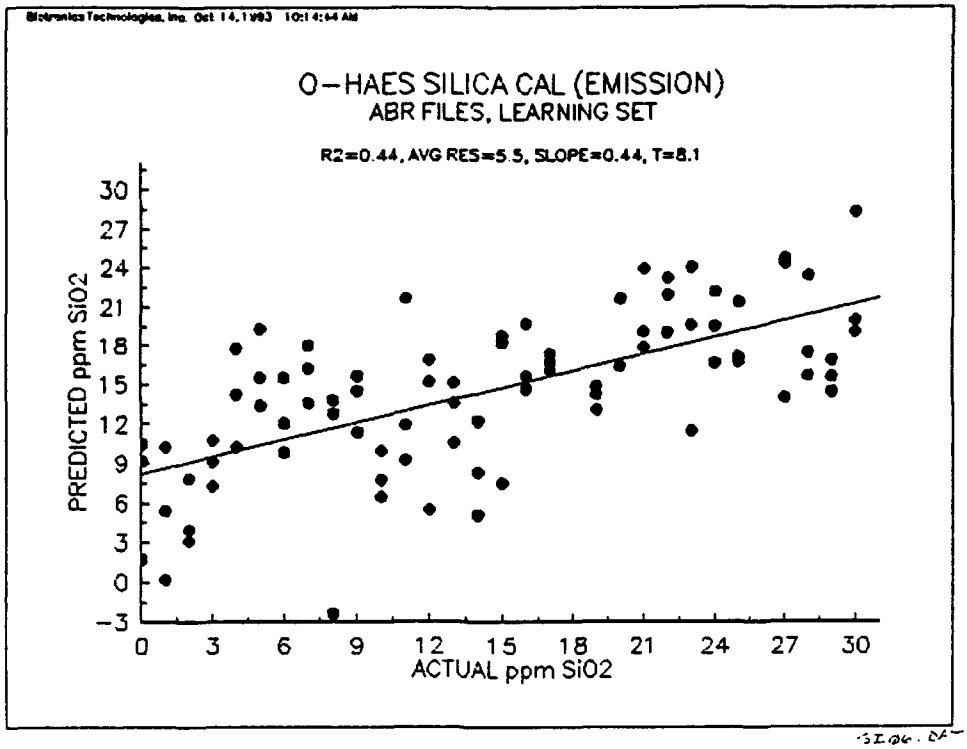

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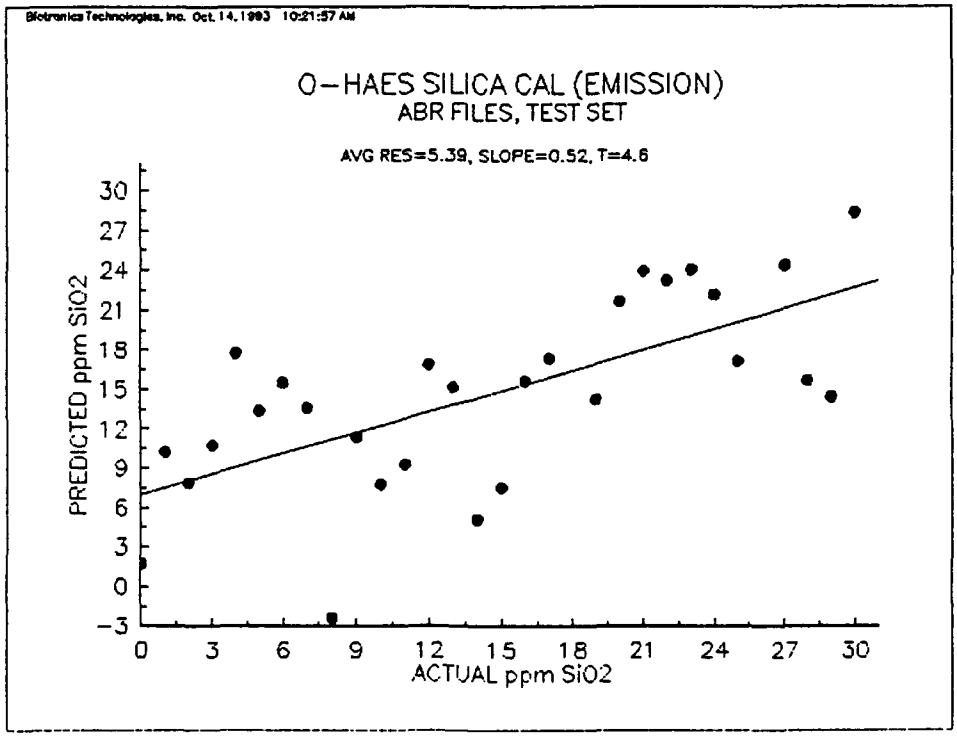

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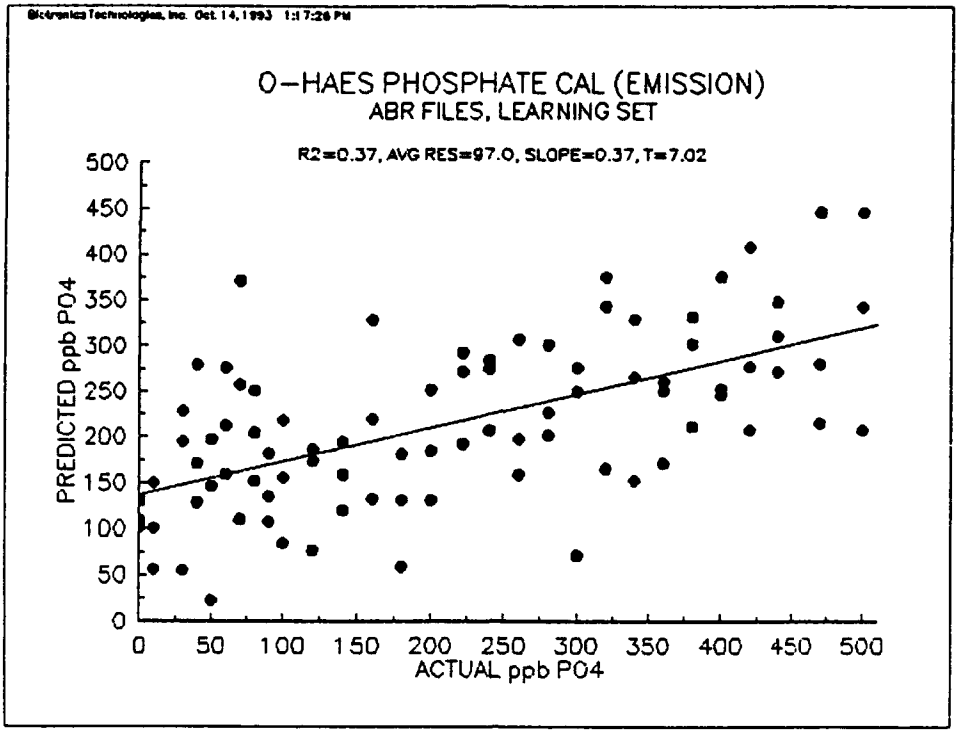

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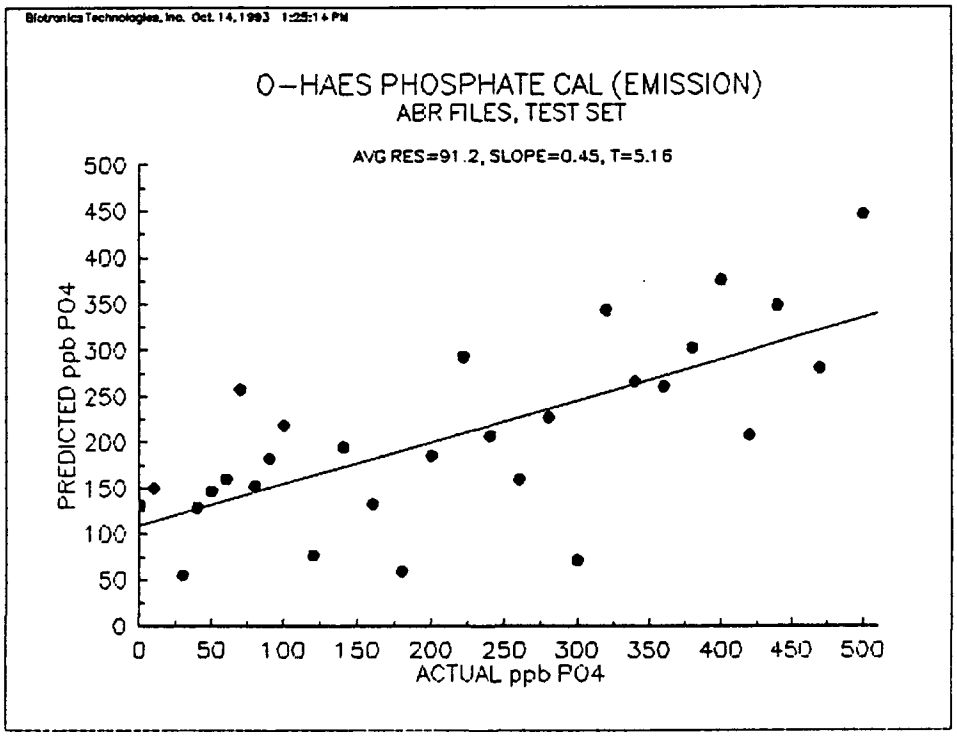


Figure 6-31.

During each cruise, samples of the bay water being tested by the OHAES were collected and evaluated for analyte concentrations by an independent, state-certified water quality laboratory. Each sample was analyzed for all ten analytes so the "actual" laboratory concentrations could be compared to the OHAES predicted concentrations. It is important to note that the laboratory concentrations may potentially err in accuracy and precision, and that this error may falsely be attributed to the OHAES instrumentation. This error could be due to improper sample handling, delays in analysis, changes in the sample due to heat, cold, or microbial digestion of nutrients in solution, chemical analysis error, and/or human error.

In fact, laboratory error required the addition of a cruise/field test in January. This was required because all the nitrate concentrations determined by the laboratory for the first three cruises were wrong because they did not take into account the salinity (i.e., high conductivity or high activity) of the water (bay) sample. The laboratory used an ion selective electrode to measure nitrate concentration. This method can be used in salt water if the standards used for calibration of the ion selective electrode have similar salt/activity levels. Despite clearly indicating the source of the samples as the Chesapeake Bay, no allowance was made by the laboratory for the high conductivity. When the laboratory returned the analysis results, the nitrate concentrations were much higher than the highest figures quoted by the EPA for nitrate levels in the bay[3,4]. Because the laboratory numbers did not look correct, a test set of samples was prepared at Biotronics Technologies with varying salinity and nitrate levels to check the laboratory's ability to predict nitrate levels. With these test samples, the laboratory performed well when the salinity was zero, but could not accurately determine nitrate levels in salt water. Therefore, an alternate laboratory in Baltimore was selected. Next, a test set prepared at Biotronics Technologies was evaluated at the new laboratory. Finally, actual Chesapeake Bay water samples from the January cruise were analyzed there.

In some cases, for some analytes, laboratory analysis was also limited due to the very low concentrations of the analytes present in the bay water sample. For some analytes, for example nitrite, silica, and phosphate, the laboratory was only able to provide data for a very few samples because the majority of the samples had concentrations below the detection limit of the instrumentation and methodology being used. In addition, the concentrations of analytes in many samples remained fairly constant. The large ranges of concentration that the OHAES was originally calibrated for often were not a reality. This lack of variation in the actual concentrations made it difficult to update the original OHAES calibrations with field spectra and also made it difficult to evaluate OHAES performance.

For all field testing, the YP-686 was limited to cruising in the Chesapeake Bay. This was due to Naval Academy restrictions as well as to time available and the normal cruise speed of the YP-686 (about 10-15 knots). However, the last cruise in January was further restricted due to weather and ice. This cruise was completed entirely within the Severn River near the bay. Because of the limited test area and therefore limited range of nitrate concentrations expected, each sample was studied several times, first in its original state, and then spiked with a known nitrate stock solution to increase the nitrate concentrations in approximately 0.5 ppm steps. In addition, the samples were diluted in half to check for background independence. The diluted samples were run and also spiked with nitrate stock solution and run again.

As mentioned in Section 3, the samples being analyzed by the OHAES were taken from a on-board plumbing system that draws water from approximately 2 meters below the stern of the ship. This system also provided sample water to an on-board thermosalinograph that provided temperature and salinity information. The system worked fairly well, although the flow rate was less than optimum. However, after a deep freeze in early January, the system was rendered inoperable and could not be used for the January cruise. Instead, buckets were used to bring water into the ship's laboratory for OHAES analysis.

The cold freeze that damaged the plumbing system also caused problems in the YP-686 laboratory that caused a loss in electrical power and a subsequent loss in heat. Unfortunately, the OHAES plumbing system had some water remaining in the flow cells, and two optical windows in the short absorbance flow cell were cracked, rendering it unusable for the January field test.

The general intent of all Phase IV field testing was to first evaluate whether the Biotronics Technologies calibration algorithms would generalize to the Chesapeake Bay water. It was expected that they would not because of the different background in the bay water, especially the presence of organic compounds that were not included in the Biotronics Technologies samples and are known to absorb in the ultraviolet to visible wavelengths. Next, the Biotronics Technologies calibrations would be updated with spectral information and laboratory analyte concentrations from the first two cruises. This updated calibration would be evaluated for its prediction ability on the third cruise. As explained above, all nitrate data is from the fourth cruise. Also, due to laboratory analysis expense, only nitrate was evaluated on that cruise. Results for each analyte are explained below.

6.4.1 Phase IV Nitrate Results

As was expected, the original Biotronics Technologies calibration did not generalize to the Chesapeake Bay. Actual field sample spectra and corresponding concentrations were required to incorporate the bay background into the algorithm. An example of the difference in absorbance curves between the Biotronics Technologies samples and an actual field sample is shown in Figure 6-32. Both of the spectral curves in this figure contain nitrate at approximately 1.2 ppm. The high level of background absorbance in the bay sample raises the entire absorbance curve. Use of a reference wavelength[7] to subtract out the absorbance not caused by nitrate allows these two types of spectra to be combined in a learning set to provide a more generalized calibration algorithm. A total of 13 field samples were added to the original 90 in-house calibration samples to form the learning set. The resulting predictions for the field test set are shown in Figure 6-33. This plot shows nitrate ranging from 0.2 to 4.5 ppm; however, the original bay/river samples ranged from 0.4 to 2.6 ppm nitrate and were then spiked and diluted to expand the range being studied. In general, the comparison of actual to predicted concentrations shows excellent predictive power, including relatively low error and high tracking of nitrate concentrations in field conditions.

6.4.2 Phase IV Nitrite Results

Nitrite concentrations are typically much lower than nitrate concentrations due to oxidation or reduction of the nitrite ion. During this study, the nitrite levels were so low, they were below the detection limit (10 ppb) for all but three samples. In addition, the concentrations in three samples were right at the detection limit. These values are significantly lower than the average bay nitrite levels reported by the EPA of 20-140 ppb[3,4]. Therefore, insufficient data were available to update the original Biotronics Technologies calibration. Nonetheless, Figures 6-34 and 6-35 show the actual and predicted nitrite concentrations based on the original calibration for the short and long flow cells, respectively. On each figure, laboratory concentrations may be shown more than once because two or more OHAES reading may correspond to each laboratory reading. In waters with higher nitrite levels, based on the success achieved during the in-house calibration, the OHAES could be field-calibrated to determine nitrite concentrations if the appropriate field data were collected.

6.4.3 Phase IV Ammonia Results

The ammonia calibration was updated with field data from the first set of cruises in October. The average ammonia concentration reported by the laboratory for these two cruises was 626

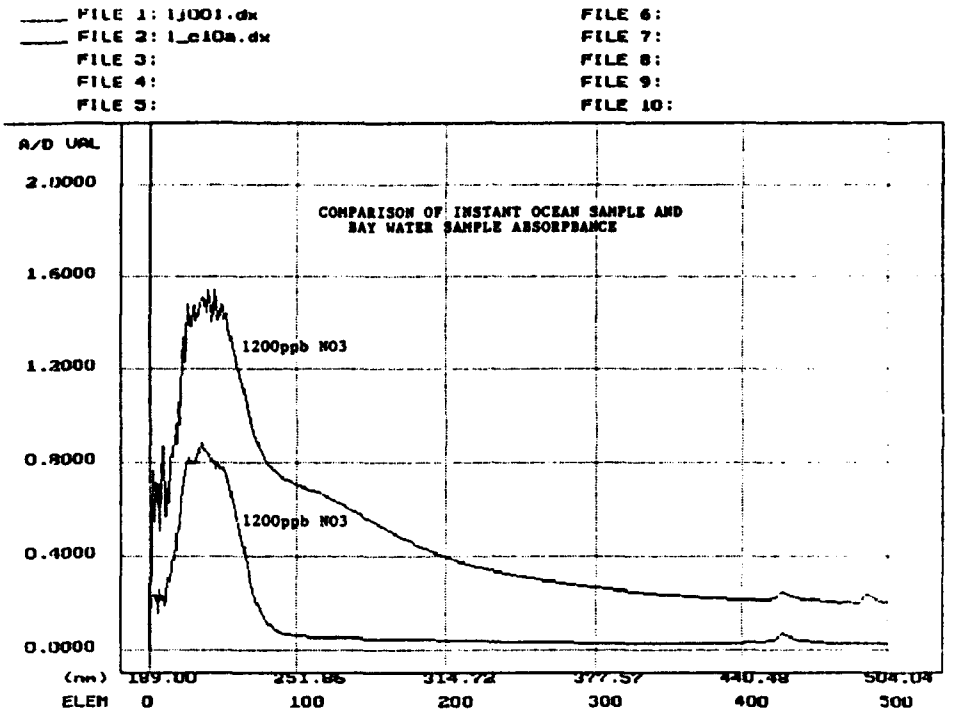


Figure 6-32. Comparison of Biotronics' sample and Bay water absorbance curves

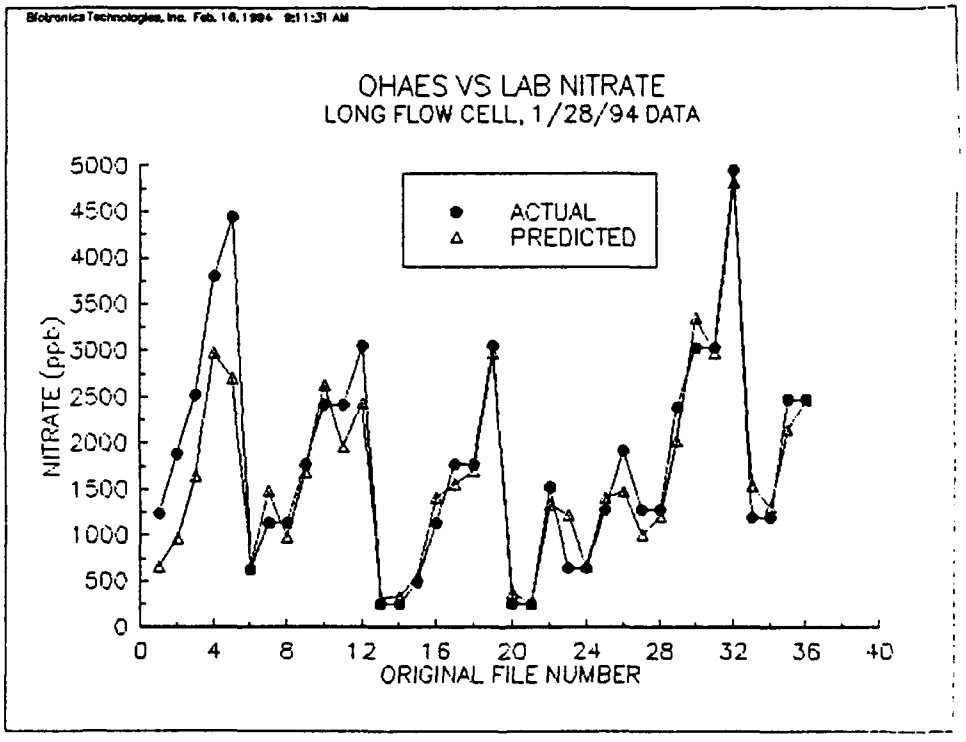


Figure 6-33.

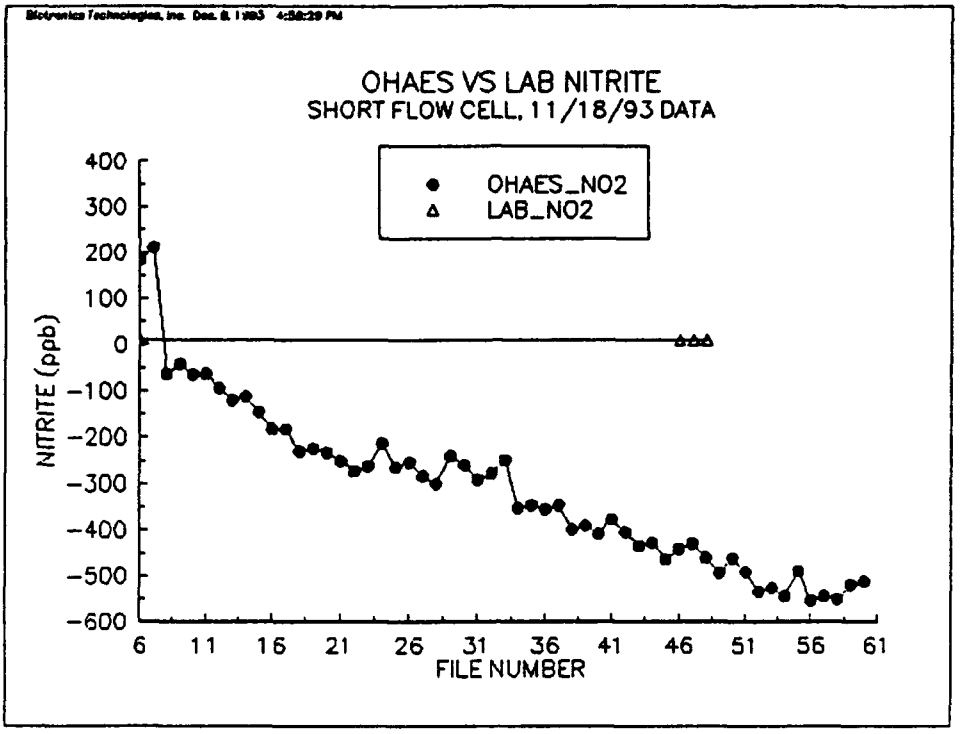


Figure 6-34.

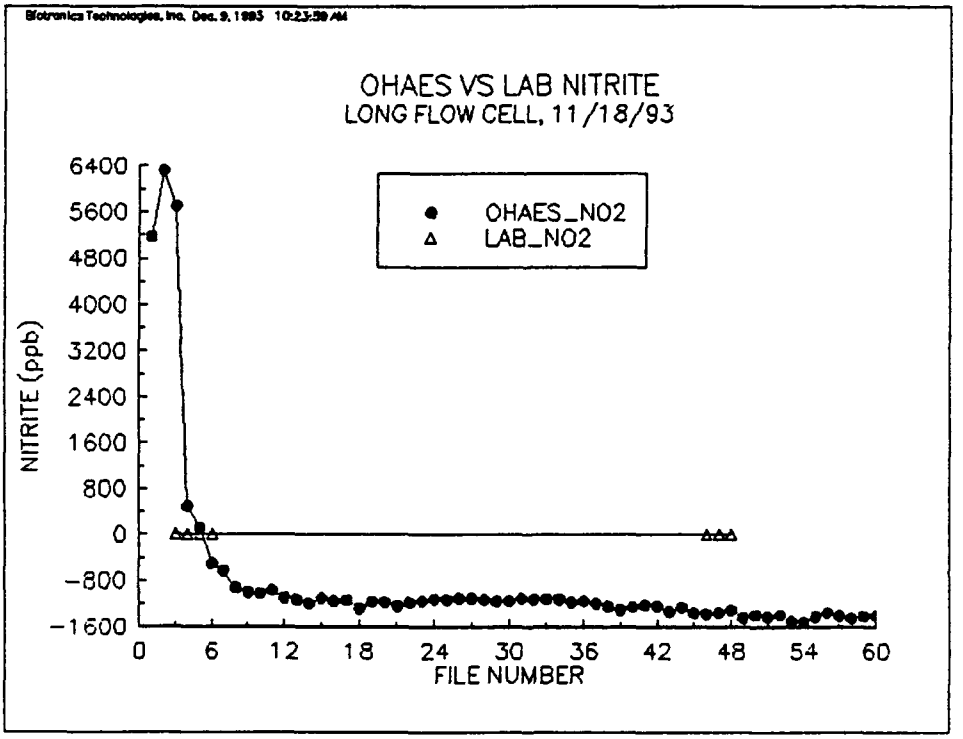


Figure 6-35.

ppb, which was significantly higher than the 35-70 ppb range of averages reported by the EPA for the Chesapeake Bay[3,4]. The subsequent laboratory data collected in November were more reasonable, with an average ammonia concentration of 50 ppb. However, the first set of data seems to have skewed the OHAES calibration algorithm, as shown in Figures 6-36 and 6-37, for the short and long flow cells. Additional experimentation with this analyte may result in a more reliable prediction algorithm.

6.4.4 Phase IV Copper Results

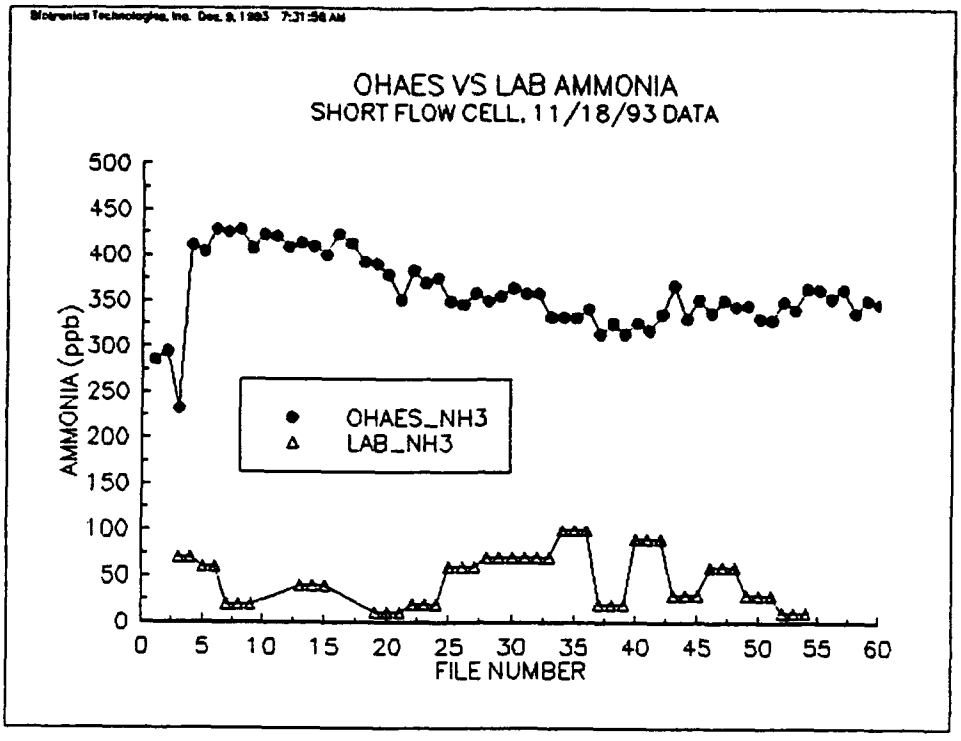
The copper results are shown in Figures 6-38 and 6-39 for the short and long flow cells. As with the ammonia, there is a notable difference between the average copper concentration from the first two cruises (115 ppb) as compared to that of the third cruise (70 ppb). In general, the short flow cell seemed to more accurately predict copper concentrations, perhaps due to the longer path flow cell allowing more absorbance from organics and nitrate and therefore not being sensitive enough to an analyte such as copper that is present at such low relative concentration.

6.4.5 Phase IV Iron Results

Iron was the only analyte for which the in-house calibration appeared to reasonably predict the bay iron concentrations. Luckily this was the case because the first two cruises only returned one laboratory sample value; the remainder were below the detection limit. The comparison of OHAES to laboratory predictions for the short and long flow cells are displayed in Figures 6-40 and 6-41. As with copper, OHAES iron predictions from the short flow cell more closely match laboratory estimates than those of the long flow cell. Likewise, the greater amount of light capable of passing through the short flow cell as compared to the long flow cell may allow better prediction of an absorbing analyte (e.g., iron) present in significantly less proportion in the solution.

6.4.6 Phase IV Calcium, Magnesium, and Potassium Results

The OHAES calcium, magnesium, and potassium predictions were compared to both laboratory values and to computed values based on the salinity recorded by the thermosalinograph on the YP-686 during the cruises. The computed salinity values were calculated by applying the "Rule of Constant Proportions" to the Chesapeake Bay waters. This rule states that "regardless of how the salinity may vary from place to place, the ratios between the amounts of major ions in the waters of the open oceans are nearly constant[11]." Because the field test was conducted in the Chesapeake Bay and not the open ocean, laboratory analysis of these analytes was also performed as an additional point of comparison and, as shown on the Figures 6-42 through 6-44, it can be seen that using the Rule of Constant proportions with bay water samples produces acceptable results. In each figure all three values, OHAES, laboratory, and calculated, are shown for comparison. The OHAES predictions are based on atomic emission spectra from the LAES portion of the system. In general, the predictions for these three analytes are all fairly close to the laboratory/calculated values with a fair amount of variability. If a moving average or some type of smoothing algorithm was added to the emission predictions, the average error would be significantly lowered, and the OHAES predictions would more closely match the laboratory/calculated values for calcium, magnesium, and potassium.



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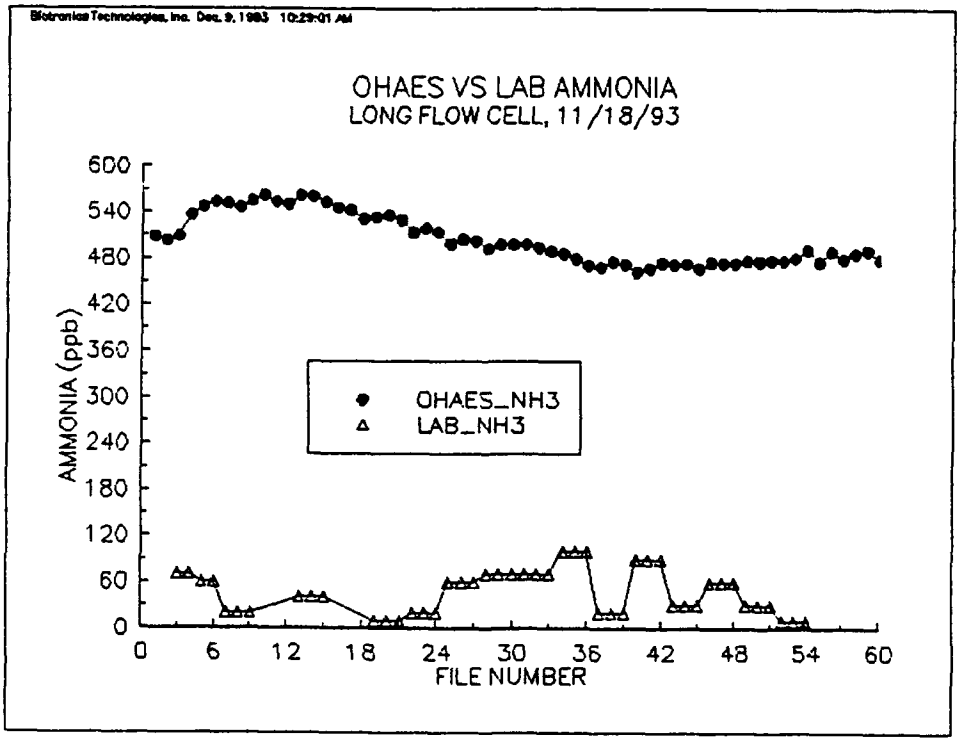

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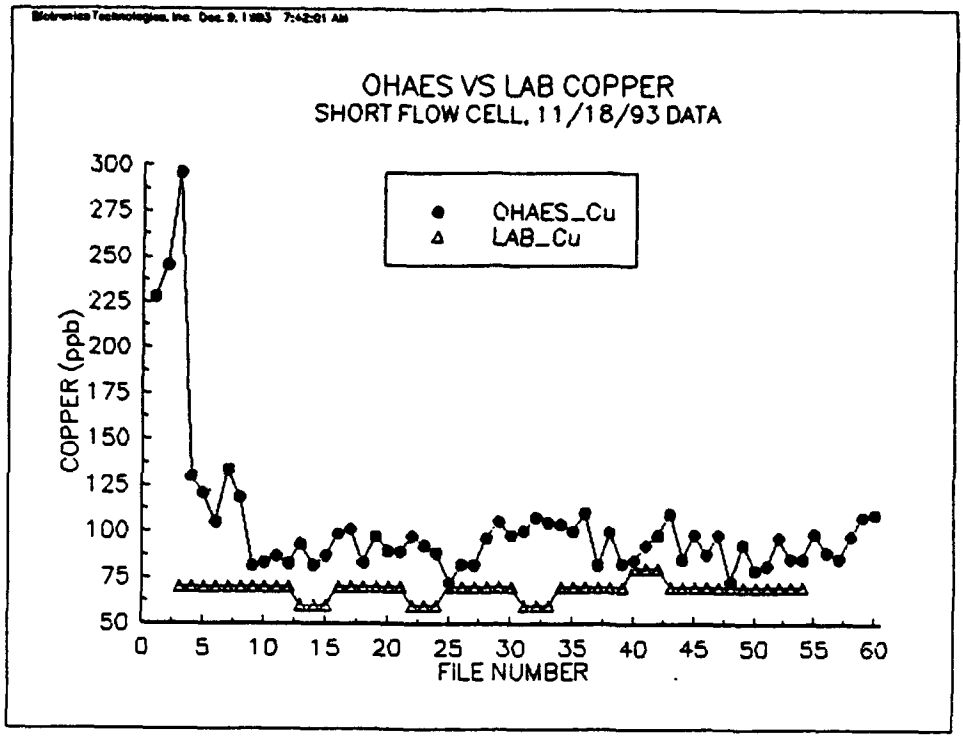

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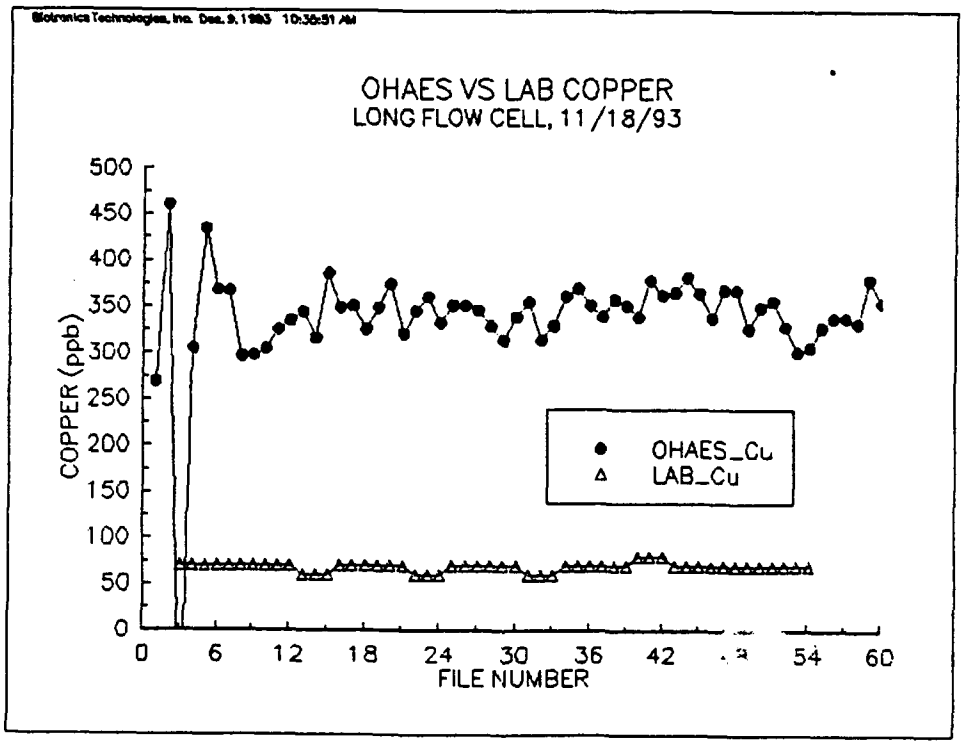

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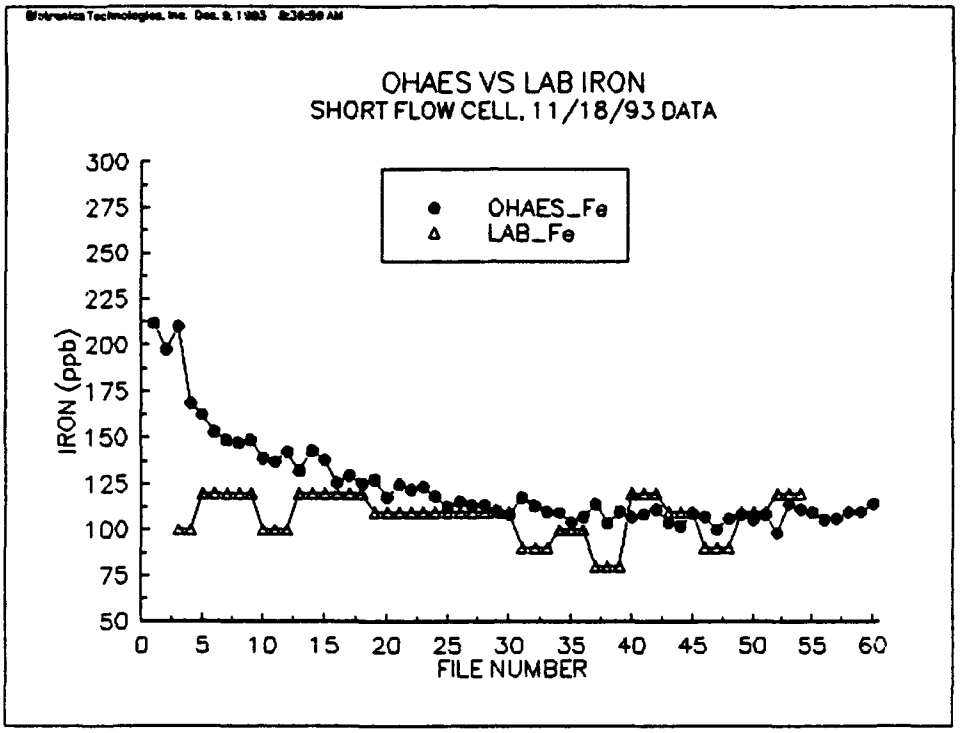

Figure 6-40.

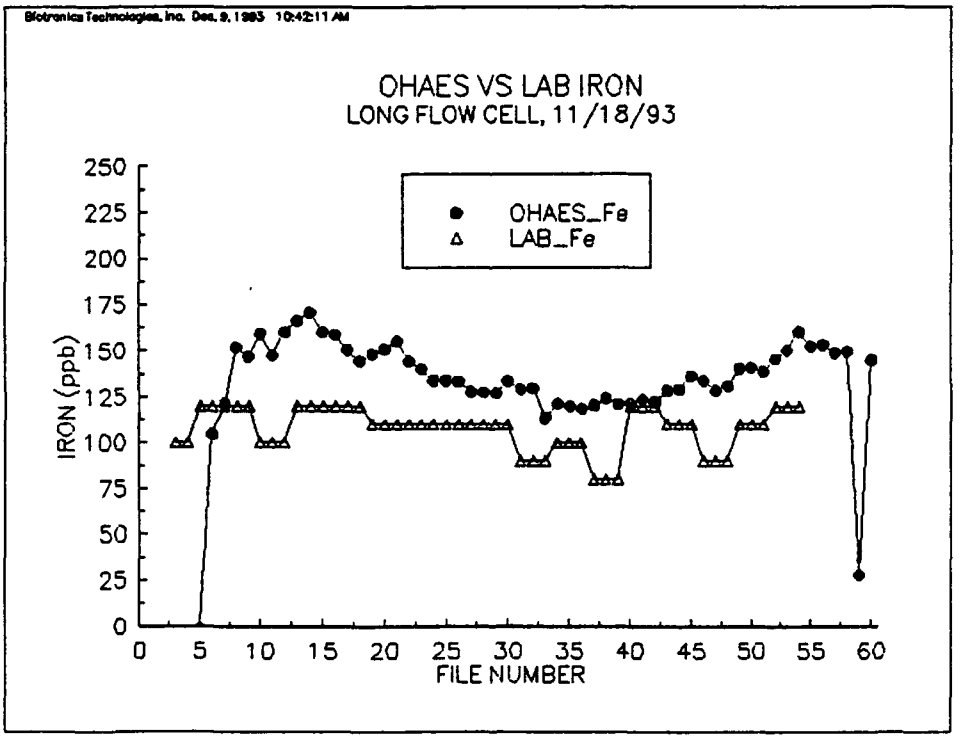

Figure 6-41.

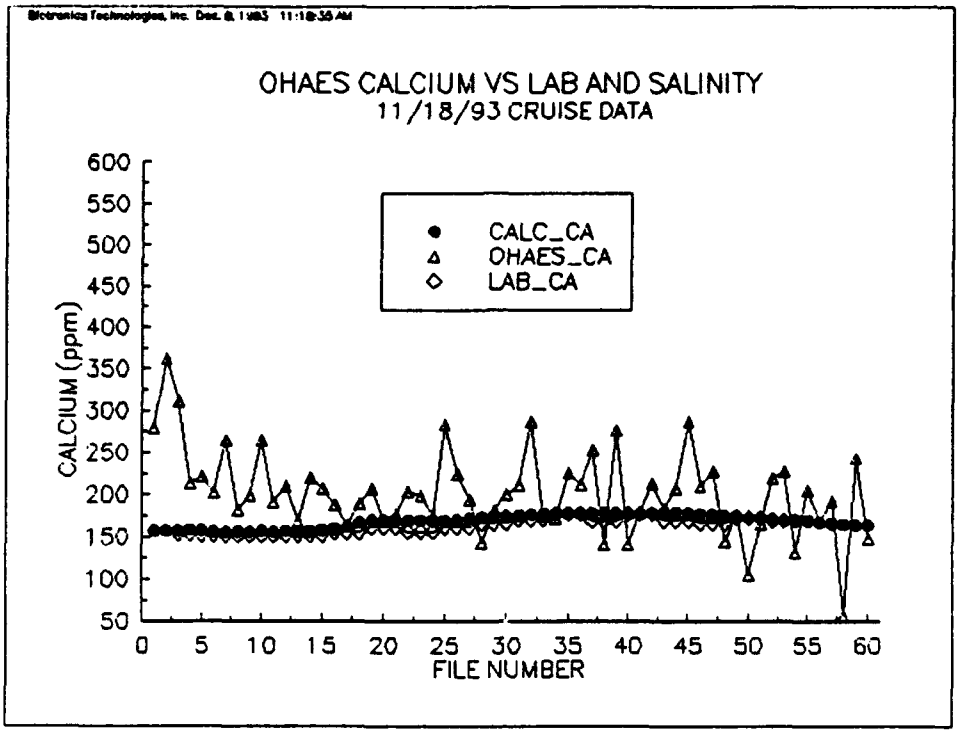

Figure 6-42.

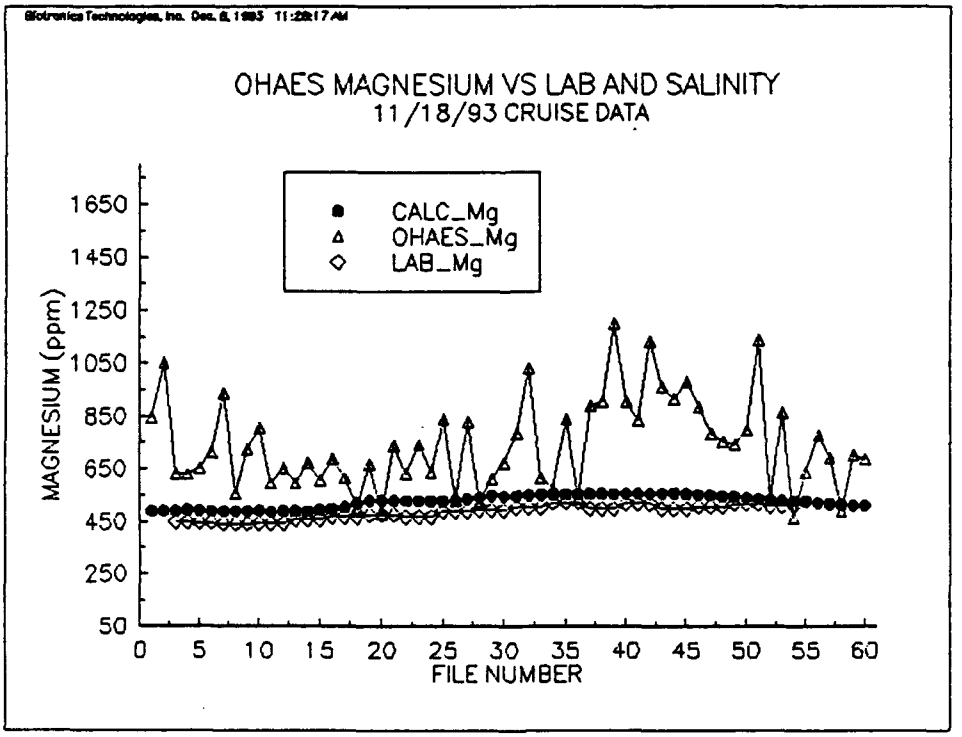

Figure 6-43.

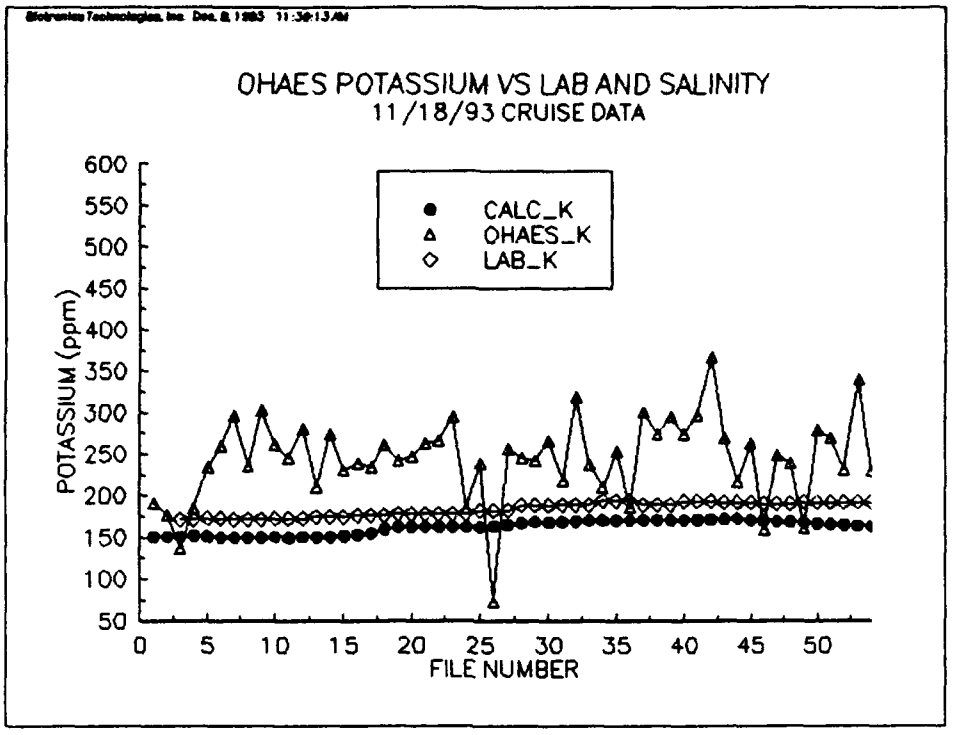
OHAES POTASSIUM VS LAB AND SALINITY
11/18/93 CRUISE DATA
CALC_K
OHAES_K
LAB_K
POTASSIUM (ppm)
600
550
500
450
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
40
45
50
FILE NUMBER

Figure 6-44.

6.4.7 Phase IV Silica Results

Silica was another analyte for which all laboratory values from the first two cruises were below the detection limit. The third cruise only provided four laboratory data points, all very close to the detection limit of 0.5 ppm. Because of the lack of data, the original in-house calibration could not be adjusted for field conditions. Figure 6-45 shows the OHAES predictions based on the original calibration and the few laboratory values that were available. The OHAES silica predictions were based on emission spectra.

6.4.8 Phase IV Phosphate Results

As with silica, all laboratory values for phosphate from the first two cruises were below detection limits so the original calibration could not be updated. Data from the third cruise showed very low phosphate concentrations in a narrow range from 10-30 ppb. The order of magnitude of phosphate does closely correspond to that seen during EPA studies when the average across the bay ranged from 10-50 ppb. The OHAES predictions, which are based on emission spectra and the in-house calibration, and the comparable laboratory values are shown in Figure 6-46. The high variability of emission predictions is quite evident.

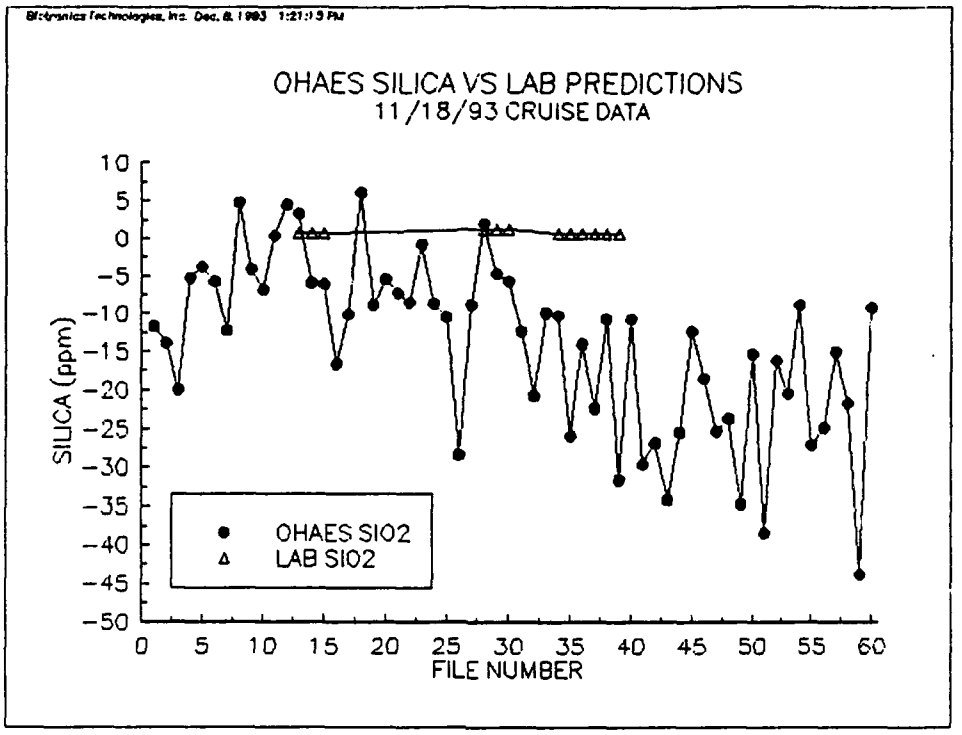

Figure 6-45.

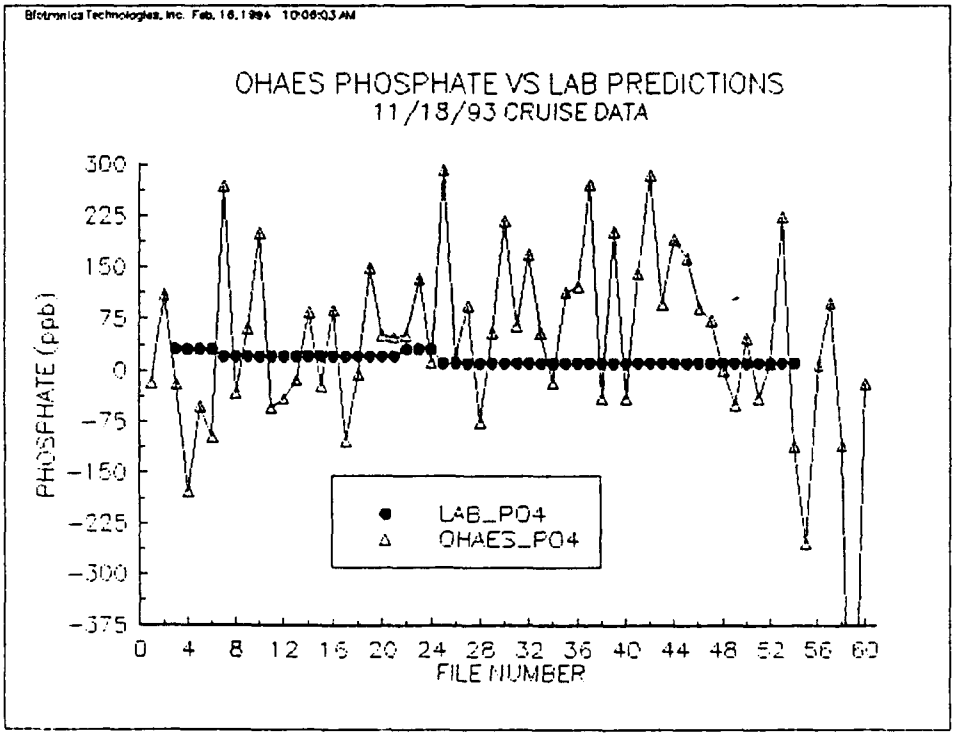

Figure 6-46.

7. FINDINGS AND CONCLUSIONS

Biotronics Technologies has successfully completed the four contracted phases of this project. By first studying the reagentless spectra of a variety of ocean compounds in different backgrounds, and then designing, manufacturing and finally testing a one-of-a-kind, Oceanographic Hybrid Absorption/Emission Spectrophotometer (OHAES), all stated objectives have been accomplished. Of the compounds (analytes) originally given for study by Naval Operations, the concentrations of some can be reliably predicted by the OHAES, while others require additional work to be detected via this reagentless spectral methodology.

The major indicator of the final performance of the OHAES is based on comparisons of the OHAES concentration predictions to concentrations determined by an independent laboratory. Unfortunately, there can be error in laboratory analysis due to sample handling, analysis technique, delays in analysis, or human error. As the final overall evaluation of OHAES performance is made, the possibility of laboratory error must be considered. In addition, for many of the analytes being evaluated there is very little data currently available for comparison or the data does not cover sufficient concentration range to allow for the evaluation of the calibration's ability to track with changes in concentration. Lacking this data and/or a good variation in the data range, it is difficult to thoroughly evaluate the OHAES performance.

Despite these limitations in performance evaluation, it was shown that the OHAES can reliably predict nitrate concentrations in open bay waters. This is a great achievement because high nitrate levels can be hazardous to the environment[4]. The OHAES could be used to monitor open waters for changes in nitrate concentrations in more detail than is currently possible by collecting grab samples and taking them to a laboratory for evaluation. Because OHAES absorption readings can be performed as often as every three to four minutes, if desired, a detailed portrait of nitrate concentrations could be easily produced on a weekly or even a daily basis. This could provide valuable information when evaluating the health of a body of water or comparing the change in the water condition over time. Additional testing in the Chesapeake Bay as well as in rivers, lakes and the open ocean would help to verify the OHAES performance in other water backgrounds. It is possible that varying background components in different waters and perhaps even seasonal variations in the same body of water would require some update to the calibration. In this case, it would be a simple matter to collect four or five samples for which absorption scans were made, analyze these samples for nitrate concentration, and then, based on the spectral data from these samples, update the slope and constant in the calibration algorithm eigenvector found from rotated principal components analysis.

Unlike nitrate, OHAES prediction of nitrite concentrations in open waters was not demonstrated during this project. However, during the simulated field test at Biotronics Technologies, a very good nitrite calibration was achieved that successfully generalized to a separate test set. The major reason that prediction of nitrite concentrations in open waters was not achieved during this project was because well over 95% of the samples collected had nitrite levels below laboratory detectable limits. There was nothing to compare to the OHAES predictions! The few data points available were insufficient to both update the calibration for field conditions and to perform an evaluation comparison. Additional testing, perhaps during a different time of year or in different waters, might provide a measurable amount of nitrite so the OHAES could be evaluated in this area. Finally, a different approach to monitoring nitrite concentration might be appropriate; instead on predicting an absolute concentration, the OHAES could be configured to determine when the nitrite levels rose above some preset (and determinable) limit. Given the previously noted low levels of nitrite, this approach may prove to be more useful than monitoring specific levels of this analyte.

OHAES predictions of copper and iron concentrations in open waters appeared to be in the appropriate range when using the short absorbance flow cell. However, lack of variation in the data did not allow an evaluation of the calibration's ability to track with changes in iron and copper concentration. In addition, the relatively poor performance of iron during the simulated field test/calibration completed at Biotronics Technologies, makes for low expectations of good iron concentration predictions in more widely varying open waters. Nonetheless, given the reasonable performance during this field test, additional studies of iron and copper are warranted.

Concentrations of three of the emission analytes, calcium, magnesium, and potassium, were satisfactorily predicted by the OHAES. Although the field test plots show a large amount of variability in these predictions, smoothing functions could be applied to take a moving average that would more closely compare to the laboratory values. Such smoothing functions are commonly used in a variety of instrumentation. Although OHAES predictions for calcium, magnesium, and potassium concentrations were fairly close to the actual concentrations, unfortunately, as for copper and iron, there is not much variability in the ranges observed. A field test that covered a larger territory up stream and out toward the ocean where a greater range of salinities exists is necessary to determine how the OHAES would perform given a greater range in the concentrations of these analytes. In fact, there are simpler methods for measuring calcium, magnesium, and potassium (for example using the salinity and the Rule of Constant Proportions as described in Section 6). Despite this, part of the purpose of this project was to determine the feasibility of using liquid atomic emission spectroscopy to study oceanography; this has been accomplished. As the LAES technology continues to be improved, the measurement of additional analytes at lower relative concentrations should be attainable. Specifically, hazardous metals such as zinc and chromium have been detected with the current LAES technology. While not included as part of this project, these metals and others such as cadmium, mercury and lead should be included in future studies of LAES capabilities.

Reagentless absorption and emission spectroscopy did not prove to be an effective means for measuring concentrations of ammonia, silica, or phosphate in open waters. For silica and phosphate, there was a very limited amount of field data for comparison, but based on the simulated field test/calibration at Biotronics Technologies, and considering the wide variations in the actual field test results, the current approach for analyzing these components with the OHAES will not achieve acceptable results. Alternate methods are being researched at this time. An option to condition the sample prior to running absorption scans has great potential for both ammonia and phosphate. In fact, by using approved standard methods[7] with the appropriate reagents for these two analytes and collecting grab samples from the water stream, the OHAES as it currently exists could be used as an absorption spectrometer to develop a standard curve and thereby predict concentrations.

Overall, given the success with determining nitrate concentrations in open waters and the potential success with similar determinations of nitrite, as well as all that was learned in the study of other analytes and in the development of the hardware and software necessary to achieve the analyte test objectives, this project has been well worth the resources expended.

8. RECOMMENDATIONS FOR FUTURE WORK

Because of the success seen thus far in this project there are many avenues of future work that could be undertaken to continue to take advantage of the OHAES potential. Some of the recommendations presented here would be fairly simple and inexpensive to implement, others are major undertakings that would require more resources.

8.1 Advanced Field Testing

Additional field testing of the OHAES is recommended to expand the confidence level in the instrument's prediction capability for nitrate and to gather sufficient data that varies over a range of concentrations to definitively prove the instrument's prediction power for nitrite, copper, iron, calcium, magnesium, and potassium. A variety of different waters, including rivers, lakes, oceans, and estuaries, should be analyzed so a larger variety of analyte concentrations are observed. Some of this testing could be accomplished on the same test vessel, the YP-686, perhaps in conjunction with NOAA cruises, which are full day cruises that cover a large part of the Chesapeake Bay, or with Naval Academy extended summer cruises, which cruise along the Atlantic Coast. To achieve the best results with this type of advanced field testing, a local "champion" for the system should be recruited. This individual could be a Naval Academy faculty member, a Hendrix Oceanographic Laboratory scientist/technician, or possibly a Naval Research Laboratory staff member who would take on this project. If Navy personnel were to be used for any of the test work, an OHAES instrument operation training course is required. In addition, provisions to make laboratory comparisons of concentrations is necessary.

8.2 Hazardous Metals Study

A logical expansion of this project would incorporate the heavy toxic metals into the list of chemical compounds analyzed by the OHAES. The potentially toxic metals shown below are a very important aspect of environmental water quality.

Lead	0.03 ppb
Mercury	0.03 ppb
Cadmium	0.10 ppb
Chromium	0.05 ppb
Aluminum	10 ppb
Zinc	0.3 ppb

The presence and concentration of these metals could be determined with the LAES, part of the OHAES system. In fact, zinc and chromium emission lines have already been identified with the current LAES system. The measurement ranges for these analytes begin at the natural levels of the metals noted above. Because of the sub-parts per billion levels at which these analytes are present, significant improvements in instrument sensitivity are required. Both hardware and software changes as well as alternate pattern recognition techniques would be part of this effort. In addition, laboratory work with each of the metals would be required.

8.3 Submersible/Towable OHAES

A return to the concept of a submersible, towable, chemical analyzer proposed earlier could provide chemical analysis information at various depths without the need to bring the water on-board the test vessel. This minimizes the possibility of contamination or biological or chemical changes that might

occur with other sampling methods. Redesigning the OHAES system into a towable package would require miniaturization of several components, re-engineering of the electrical and communication systems, and possibly changing some of the materials of construction. Of course, finding the proper tow body would be the first step.

8.4 Remote Buoy-Mounted OHAES

Another alternate approach to using the OHAES capability to predict chemical concentrations in open waters would be to mount a remote spectra gathering instrument on a buoy to monitor changes in water chemistry at one or more specific locations. This could be useful in monitoring daily, tidal, and seasonal changes and possibly determining sources of environmental pollution. As with the towable system, some miniaturization might be necessary, as well as re-engineering the power and communication systems and changing some of the materials of construction.

8.5 Shipboard Water/Wastewater Analysis Application Study

A major potential application of the OHAES system relates to on-line analysis of water and wastewater quality aboard Naval vessels. Boiler water analysis in nuclear power plants is a particularly important example. Cooling water analysis to allow for on-line control of water treatment is a second example. A demonstration project in this area could result in better quality control of shipboard water supplies.

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APPENDIX A

OHAES SYSTEM SPECIFICATIONS

OHAES SYSTEM SPECIFICATIONS

1. General Specifications

1.1 Operating Principle

The OHAES system is a hybrid system that uses both absorption and atomic emission spectrometry. For absorbance, light from a single-beam flash lamp is transmitted to the absorbance flow cells via a bifurcated fiber-optic cable. For atomic emission, solution in the emission flow cell is electrically excited by a voltage potential across gold electrodes. The light from each flow cell is collected by a fiber-optic cable and transmitted to a fixed holographic grating and linear photodiode detector array.

1.2 Operating Range

200 - 800 nm

1.3 Modes of Operation

1. On-line

   - Reads samples (absorption and emission)
   - Calculates concentrations
   - Displays concentrations on video monitor
   - Stores concentrations in computer data file

2. Off-line

   - Instrument calibration
   - Instrument standard (zero)
   - Setup of on-line parameters
   - Grab sample and other experimentation

1.4 Analytical Algorithms

1. Multiple-variable stepwise regression
2. Rotated principal components
3. Total energy normalization
4. First derivative preprocessing

2. Inputs

2.1 Physical

Calibration/test samples may be added through a removable funnel, or the flow may be plumbed in from a continuous water flow using the fixtures on the lower front of the rack. See Figure A-1 for the OHAES Flow Diagram.

OHAES FLOW DIAGRAM

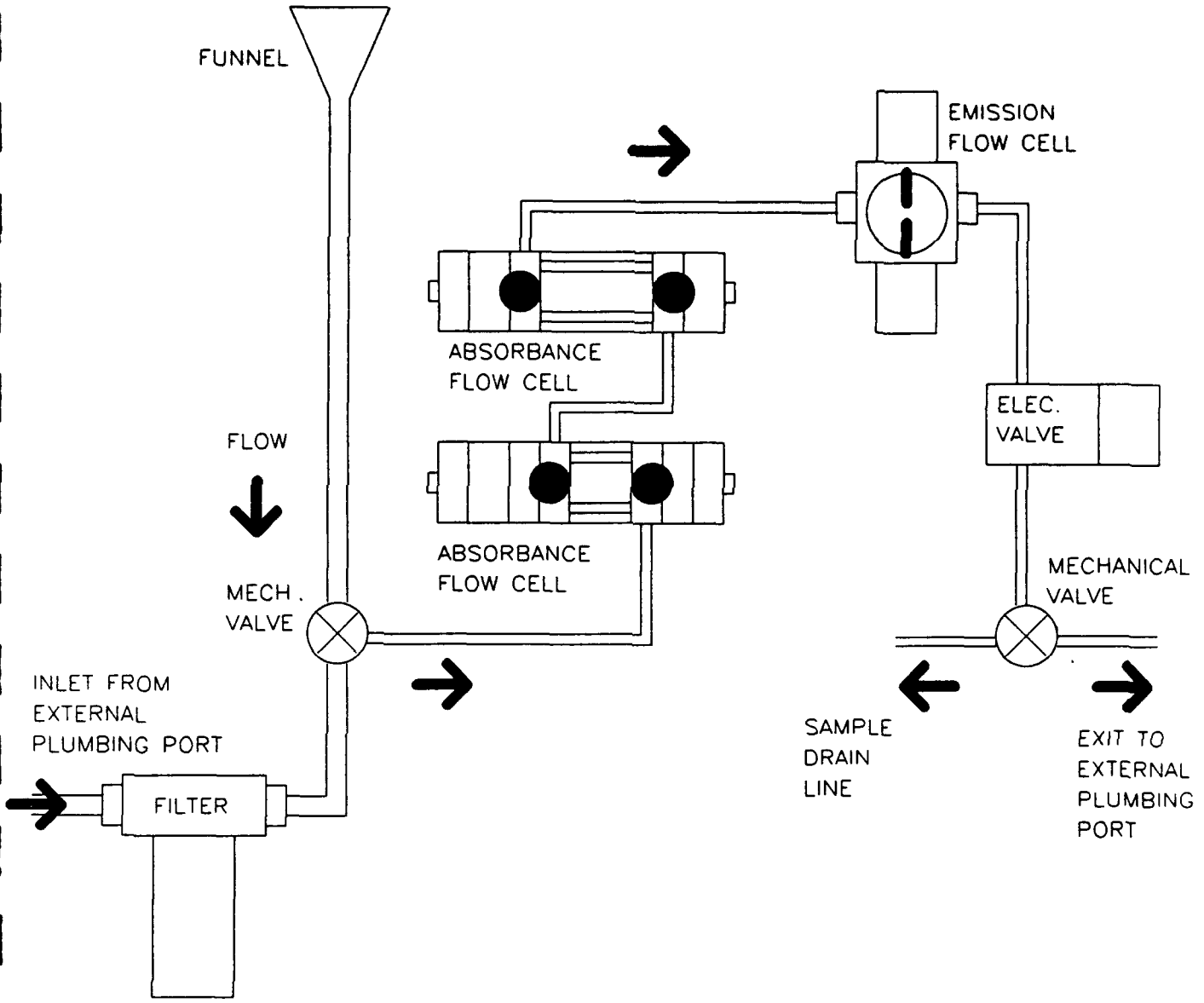


Figure A-1

2.2 Software

Instrument control software is menu driven and may be operated from the keyboard or by using the mouse. Default configuration files are included, and the instrument may be operated continuously on-line or at specific times as selected by the operator.

3. Outputs

3.1 Video Monitor

After each on-line run, concentrations are calculated and displayed on the video monitor. When operating off-line, the computed concentration file may be reviewed after a run by using the editor included with the software.

3.2 Data Files

The operator can configure the instrument computer to save the raw spectra data file and the predicted analyte concentration data files. These files can be named by the operator or a default name will be assigned by the computer. In addition to the raw spectral data, various intermediate data files may be saved for later analysis.

4. Hardware

The OHAES components are mounted in a standard 19" rack with a footprint of 24" x 33". The rack is 71" tall (including eye-rings). The total instrument weight is estimated at 425 lbs. The "Analyzer" half of the instrument includes the computer, video monitor, keyboard, and circuit boards. The "Optograph" half of the instrument includes the spectrograph, the photodiode array, the flow cells, the flash lamp, and the emission power supply. All the optograph components are mounted on one removable shelf. In addition, the emission power supply is mounted on rails and can be removed from the optograph shelf if desired. (Refer to Figures A-2 and A-3 for layout of OHAES optograph shelf.)

4.1 Light source

- Xenon flash lamp, 7 watts (200 - 800+ nm)
- Life expectancy > 2 years

4.2 Spectrograph

- CP200 off-the-shelf from Instruments SA, Inc.
- Fixed-imaging holographic grating
- 200 - 800 nm

4.3 Detector

- Self-scanning silicon photodiode array
- 1024 pixels

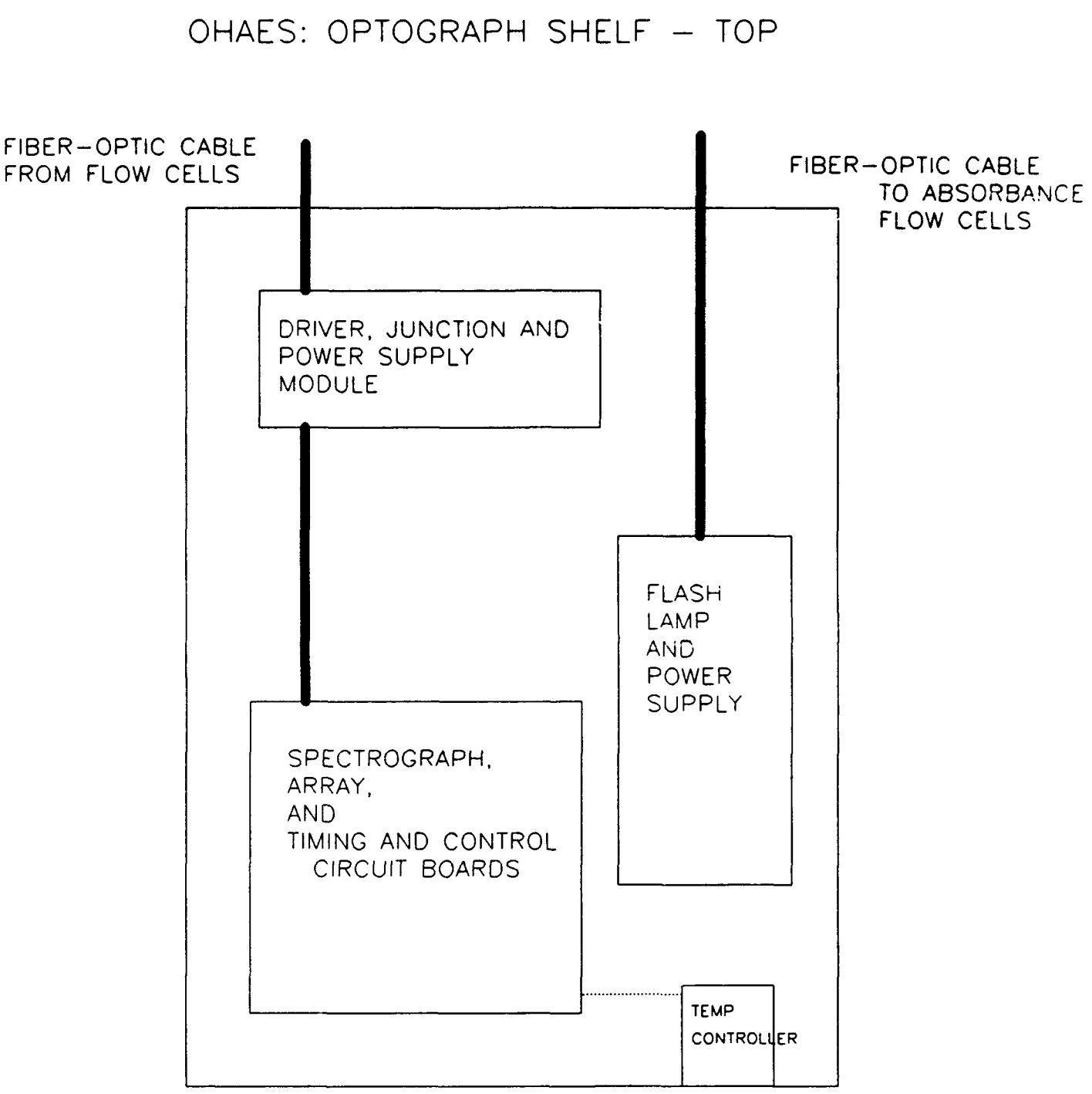

OHAES: OPTOGRAPH SHELF – TOP
FIBER–OPTIC CABLE FROM FLOW CELLS
FIBER–OPTIC CABLE TO ABSORBANCE FLOW CELLS
DRIVER, JUNCTION AND POWER SUPPLY MODULE
FLASH LAMP AND POWER SUPPLY
SPECTROGRAPH, ARRAY, AND TIMING AND CONTROL CIRCUIT BOARDS
TEMP CONTROLLER

Figure A-2.

OHAES: OPTOGRAPH SHELF – BOTTOM

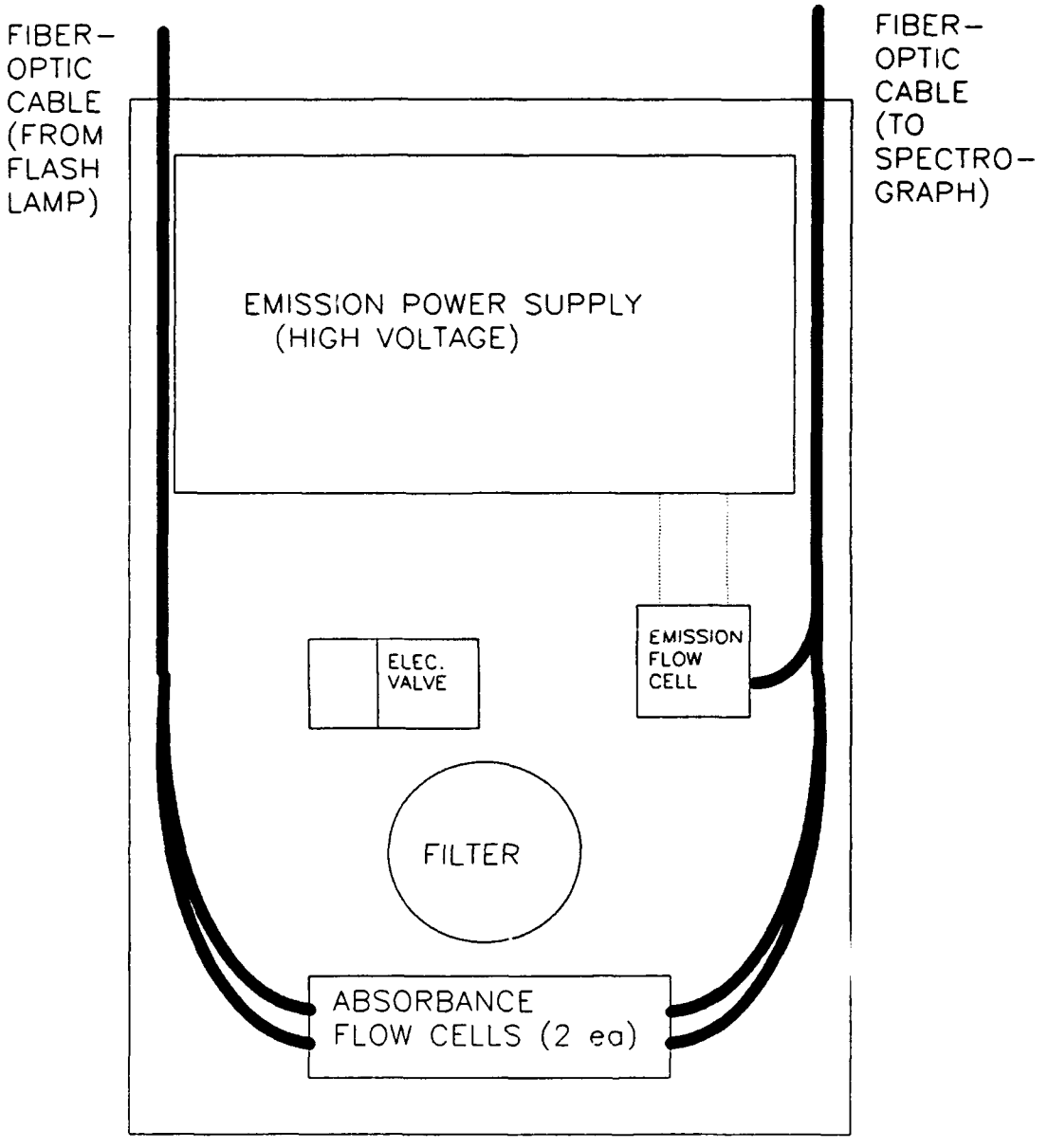


Figure A-3.

4.4 Computer

- 386DX, 33 MHz, with coprocessor
- 3.5" floppy, 85MB hard drive, 4MB RAM
- Microsoft mouse
- Quatech I/O board
- Industrial casing for computer and monitor to reduce hazards from shipboard operation

4.5 Emission Power Supply Control

WARNING: The emission power supply is capable of producing up to 60,000 volts. DO NOT OPERATE THE SYSTEM UNLESS PROPERLY TRAINED TO DO SO.

See Figure A-4 for a layout of the emission power supply components discussed below.

Interlock Safety System - will not allow the internal computer to power-up the emission power supply from standby (green) to armed (red) when the optograph shelf is in the extended position. The safety system can be overridden by an operator pushing the (square) red button to on.

Green Standby Light/Button - indicates emission power supply is in standby (low voltage) condition. This button is located on the optograph shelf front panel.

Red Armed Light/Button - indicates emission power supply is armed and ready to read (high voltage). To take the emission power supply off of armed status, press the green standby light/button. These buttons are located on the optograph shelf front panel.

Main Circuit Breaker/Power-On Switch - removes all power from emission power supply. This switch is located on the silver emission power supply and is accessible through the back rack access door.

Current Control Knob - adjusts arc current from 0 to 10 amps. This knob is located on the silver emission power supply and is accessible through the back rack access door.

Fuses - 2 amp fuses: one for the spark circuit, one for the arc circuit. These are also located on the silver emission power supply accessible through the back rack access door.

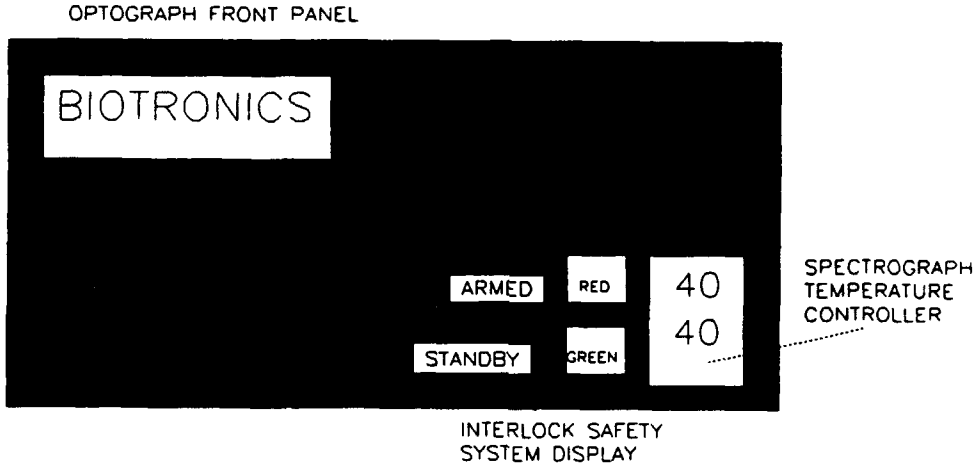
Auxiliary Spark Gap Control - The spark gap may be manually adjusted. It is preset by Biotronics Technologies for optimum spectral output. Varying the gap will vary the voltage potential seen by the electrodes. A small silver panel must be removed to adjust the electrodes, this panel is on the right side of the emission power supply.

4.6 Temperature Controller

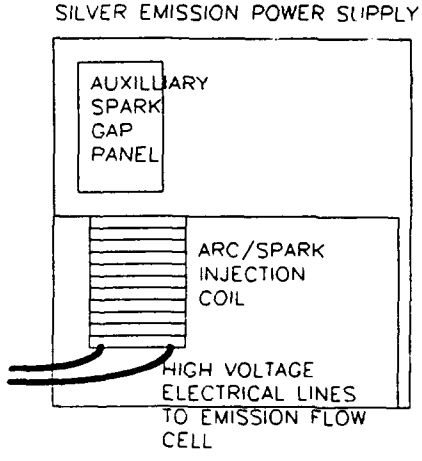
- Fuzzy logic/PID controller maintains spectrograph enclosure temperature at 40°C (or as desired)
- Constant spectrograph temperature reduces optical changes due to expansion or contraction of the spectrograph or its grating and is required for optimum performance
- Controller Brand: PYX-4 from Total Temperature Instrumentation
- Manufacturer's manual for the temperature controller is included with the OHAES system.

EMISSION POWER SUPPLY COMPONENTS

VIEW FROM FRONT OF INSTRUMENT RACK



VIEW FROM RIGHT SIDE OF INSTRUMENT RACK



VIEW FROM BACK OF INSTRUMENT RACK

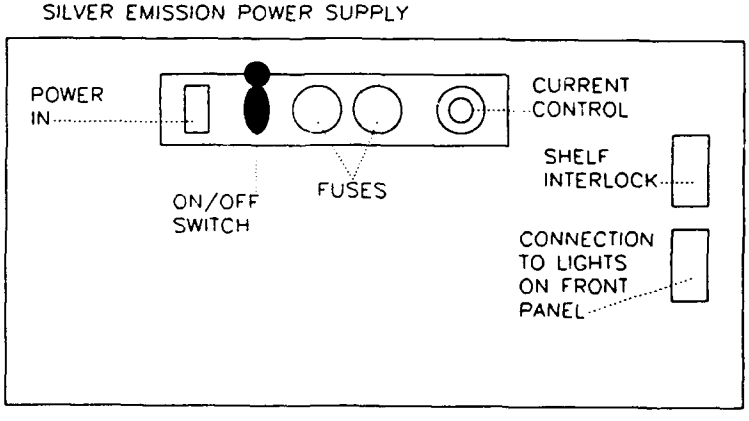


Figure A-4.

4.7 Rack/Enclosure

- NEMA 12
- Provides protection from spraying water, dust, and dirt
- Rack opening is for standard 19" rack-mounted equipment.
- Dimensions: 24" w x 33" d x 71" h
- Weight: Approximately 425 lbs
- Plumbing input and output fitting size: 1/4" FNPT ports

4.8 Power Requirements

- Standard 120 V ac, 50/60 Hz, 15 amps
- One input line provides current to surge-protected distribution strip.
- Internal power setup so the temperature controller and heating system will operate with the instrument powered off. To turn off the heating system, you must unplug the appropriate line within the upper rack enclosure.

4.9 Flow Cells

- Two absorbance flow cells and one emission flow cell are included in the OHAES system.
- Absorbance flow cells may be removed for cleaning without disturbing the optics.
- Four path length options are provided: 10 mm, 25 mm, 50 mm, and 100 mm. Two different path lengths (25 mm and 100 mm) are installed in the two absorbance flow cells.
- Absorbance flow cells have an electrical shutter as part of the flow cell system so light from the flash lamp can be directed through either one or the other flow cell as desired.
- Emission flow cell electrodes: 18 kt gold, 1.5 mm diameter, expected life span of 6 months (depending on number of readings)
- Fluid port connector fitting size: 2 1/8" FNPT fluid ports
- Fiber-optic connectors: SMA half-length adapters for type 905 SMA cable connectors

4.10 Fiber-Optic Cables

WARNING: The fiber-optic cables are extremely fragile! They are easily damaged and are very expensive to replace. They must not be bent or coiled tightly. Cables should only be handled by trained personnel.

Specially designed fiber-optic cables have been installed in this instrument. A bundled, bifurcated cable transmits light from the flash lamp to the absorbance flow cells. A trifurcated cable transmits light from the two absorbance flow cells and the emission flow cell to the spectrograph.

5. Performance

5.1 Accuracy

Based on the Biotronics Technologies calibration of the system prior to shipment, an estimate of the average error for the analytes is shown below.

		AVG ERROR	RANGE
- Absorbance:	Nitrate (NO_3)	45 ppb NO_3	10-5000 ppb
	Nitrate (NO_2)	42 ppb NO_2	10-500 ppb
	Ammonia (NH_3)	95 ppb NH_3	10-500 ppb
	Copper (Cu)	8 ppb Cu	0-50 ppb
	Iron (Fe)	7 ppb Fe	0-50 ppb
- Emission:	Calcium (Ca)	60 ppm Ca	50-600 ppm
	Magnesium (Mg)	186 ppm Mg	300-1800 ppm
	Potassium (K)	65 ppm K	50-600 ppm
	Silica (SiO_2)	5 ppm SiO_2	0-30 ppm
	Phosphate (PO_4)	91 ppb PO_4	0-500 ppb

5.2 Spectral Resolution

Approximately 0.6 nm

6. Environmental Requirements

Ambient Temperature - 1 °C to 38 °C

7. Sample Requirements

7.1 Sample Flow

To be determined

7.2 Sample Temperature

To be determined (expecting 10 - 28 °C)

7.3 Sample Pressure

To be determined

7.4 Sample Optical Transmission

To be determined

8. Safety Features

8.1 Emission Interlock System

- The emission power supply interlock system will not allow the high voltage arc/spark combination to be emitted when the shelf is pulled out of the rack. However, power is NOT removed from the system in this configuration.
- This feature may be overridden by a trained operator.

8.2 Ultraviolet Light Protection

The xenon flash lamp assembly has been covered by a shield to block ultraviolet light emission. Ultraviolet light poses a danger to individuals' eyes and, with prolonged exposure, to skin.

APPENDIX B

GLOSSARY

GLOSSARY

Disclaimer - not universal definitions - defined only as applicable to OHAES system.

Absorbance

A relative measurement of the amount of light passing through a sample as compared to some baseline measurement of the same light.

Absorption

Light passing through a medium (in this case the sample solution) is absorbed by the compounds in the medium. The light that exits the medium differs in wavelength and intensity from the light that entered the medium.

Absorption Spectrum

A graphical display of the intensity of the light that remains after passing through a medium (i.e., the light that has not been absorbed by the solution) and has passed over the OHAES system spectrograph's wavelength range.

Analytes

Chemical components in the solution being analyzed.

ASCII File

File type that is easily read and edited with may common editors.

Beer-Lambert Law

States the total light absorbed is proportional to the light path length and the concentration of the absorbing component, with a constant absorptivity coefficient defining the absorptivity of the media.

Binary File

Data file whose contents are only recognizable by the applications program. The original spectra data are collected as binary files and later changed to ASCII file with a JCamp format. Binary files are required when using the "plotraw" graphing program. The standard default binary filename has an extension of "bin".

Calibration

A process whereby a number of samples of known concentration are read by the OHAES instrument so preprocessing and mathematical analysis can be accomplished. The calibration results will allow the prediction of unknown analyte concentrations for samples.

Calibration Algorithm

Best mathematical model (based on correlation, tracking, and error) for matching analyte concentrations to spectral information using various wavelengths (variables). Once determined, the calibration algorithms are stored in the computer memory and are used to predict subsequent sample analyte concentrations.

Calibration Set

Refers to a set of solutions of known concentration and the data generated by reading those solutions on the OHAES instrument. These data are used to recognize a mathematical pattern from which predictions of concentrations for other solutions may be made. Also called a learning set.

Chemometrics

Refers to application of mathematical and statistical techniques to chemical analysis.

Composite Binary File

OHAES data file composed of the original raw binary data file and additional configuration and setup information including: the absorbance instrument standard (for absorbance runs), the wavelength calibration, the concentrations of analytes (if entered), the flash/arc period and on-time, pixel information, and the number of flashed and cycles.

Configuration File

Computer file that contains setup information (i.e., configuration) for the OHAES system.

Correlation

A measurement of the strength of the association between variables. The coefficient of correlation (R) equals the square root of the explained variation over the total variation.

Cycle

A cycle consists of one dark scan and one light scan.

Dark Scan

Refers to a OHAES instrument read that is done without any light source. The dark scan provides a reading of instrument background noise that can then be subtracted from a light scan to produce the final reading.

Fiber-Optic Cable

Light, small fibers that transmit light waves. Virtually immune to electrical interference (i.e., high voltages). The fibers do not radiate the signal (i.e., the light) they carry, nor are they susceptible to the acceptance of induced signals. Also, optical fibers accept a large bandwidth with very low power loss. Typically fiber optic cables are made of glass and are very fragile.

Fiber Optics

Fiber optics is the technology that allows light energy to be transmitted as light waves through an extremely small fiber to a receiver. At the receiver the light may be converted to electrical impulses.

Flash On-Time

The length of time the flash lamp is on for one flash in a scan.

Flash Rep Rate

Time between the initiation of one flash in a scan and the next flash in the same scan.

Flow Cell

(Same as optrode.) The physical chamber through which the sample or process stream flows where the light is absorbed or emitted.

Instrument Standard

Sets a baseline that OHAES absorbance readings can be measured against. For absorbance the instrument standard consists of reading distilled water with the OHAES instrument. This baseline/standard is used as a comparison for following runs (both calibration and on-line).

JCamp Data File

Refers to a type of data file with a specific format. The file is an ASCII type file with header information and x-y data including the wavelength and corresponding light intensity or absorbance.

Learning Set

See Calibration Set.

Light Scan

A scan that is performed while the absorbance flash lamp is flashing or the emission arc is arcing.

Multiple-Variable Stepwise Regression

Mathematical algorithm used to identify a pattern in the spectral data.

Optograph

Refers to subsystem of OHAES which includes the optrodes (flow cells), spectrograph, photodiode array, emission power supply and other electronics and junction boards.

Optrode

Sample flow cells through which optical measurements are made.

Pattern Recognition

Computer code and mathematical algorithms required to take data from a learning sample set and predict concentrations for a test set (or on-line).

Photodiode Detector Array

The array receives the light from the OHAES system spectrograph. For each pixel in the array (1024 for this instrument), a value related to the amount of light at the pixel is assigned. The greater the number of pixels, the greater the resolution of the range of wavelengths coming from the spectrograph.

Pixel

Refers to one detector on the photodiode array. For the OHAES there are 1024 pixels (individual detectors) on the photodiode array. The light from the spectrograph is dispersed over the range of 200-800 nm, so each pixel "sees" approximately 0.6 nm waveband. A wavelength calibration file is created by using a mercury lamp so each pixel is associated with a specific wavelength.

Precycle

Precycles are light and dark scans that are performed without data being collected before the regular cycles are initiated. The precycles allow the instrument time to stabilize before data collection begins.

Process Stream

The flow stream of the process that is to be monitored by the OHAES system.

R^2 Results

The coefficient of determination (R^2) of the variation as explained by a regression model divided by the total variation of the data. An R^2 of 1 would mean the mathematical model could perfectly fit all data points. In general, the closer to 1, the better the model fits.

Ratio to Total Energy

A mathematical processing technique whereby the intensity of light at each wavelength is divided by the sum of the light intensity at all wavelengths. This technique is recommended for analysis of emission spectra.

Reference Wavelength

A specific wavelength selected to be used in mathematical algorithms. A wavelength is usually selected because data collected at this wavelength is very predictable or constant in varying samples. It is used to normalize the data, most frequently for absorbance calibrations.

Regression Analysis

A statistical model that can predict the values of a dependent variable (in this case concentration) based on the values of one or more explanatory or independent variables stored (in this case raw or preprocessed data).

Rotated Principal Components

Mathematical algorithm used to identify a pattern in the spectral data.

Rotation Preprocessing

Mathematical algorithm used with principal components to identify a pattern in the spectral data.

Sample Cell

The center portion of the absorbance flow cells which may be removed for cleaning without disturbing the optics of the system.

Scan

Consists of a flash sequence and a data collection sequence. During the flash sequence the flash lamp or electrodes can either flash or arc (light scan) or not (dark scan).

Spectral Lines

A graphical representation of light intensity at each wavelength.

Spectrometry

The use of spectral information (absorption or emission for this instrument) to identify and or quantify the analytes in solution.

Spectrograph

Internal part of the OHAES system that receives light from the two flow cells and divides it into a range of wavelengths. For this instrument, the range of wavelengths is 200 - 800 nm.

Test Set

Refers to one or more solutions for which the analyte concentrations are predicted based on calibration algorithms derived from learning or calibration sets. Concentrations may be known (for comparison) or unknown; however, the data generated from the run may not be included as part of the learning/calibration set.

Waveband

Band of wavelengths (including a range).

Wavelength

The amount of space occupied by the progression of an electromagnetic wave. The wavelength is inversely proportional to its frequency (f), with the velocity of light (c) as the factor: $\lambda = c/f$. Typically used when referring to one or more wavelengths identified for analysis.